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EXPERIENCES WITH LAMELLAR SCLERAL RESECTION FOR RETINAL DETACHMENT: INDICATIONS AND RESULTS

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For about ten years scleral resection has been before the footlights as a treatment of retinal detachment. Recently this procedure has been used routinely. At our clinic lamellar scleral resection was first applied to one case as a trial in 1951. After that we performed this operation, choosing cases with a very poor prognosis. With the accumulation of clinical experience, however, the use of lamellar scleral resection has increased in frequency. Here we describe our personal point of view concerning the indications as well as report on the operative results obtained.

Materials and Method of Operation

The lamellar scleral resection was used in only 2 cases in 1951 and 1952, which are excluded from this report because they were a mere trial. As shown in Table 1,

Table 1. Number of Operation for Retinal Detachment During the Period from 1953 to 1959

	Number of Cases	Number of Eyes	Number of Operation	Scleral Resection		
				Number of Eyes	Number of Operation	Percentage of Total Number of Operation
1953	44	46	55	2	2	3.6
1954	66	67	97	4	4	4.1
1955	71	71	102	4	4	3.9
1956	65	65	85	5	5	5.9
1957	70	71	101	18	20	19.8
1958	91	92	121	20	20	16.5
1959 Jan. to June	41	41	55	15	16	29.1
Total	448	453	616	68	71	11.5

during the period from January 1953 to June 1959, 616 operations were performed on 453 eyes with retinal detachment. Among these, 68 eyes underwent 71 lamellar scleral resections (11.5% of the total operations) either primarily or secondarily. During the first several years of this period, the number of lamellar scleral resections performed was very small (3.6 to 5.9% of the total operations). Since 1957 there has been a marked increase in the use of this procedure; it accounted for 30% of the operations performed in the first half of 1959. These facts indicate that this procedure became more widely indicated, as our knowledge about lamellar scleral resection grew with the accumulation of operative experience.

The method used was almost identical with the technique described by Dellaporta, Panfique, Shapland, and Stallard; at the same time the retinal tear was closed thoroughly by diathermy.

Indications

It is generally accepted that eye-shortening operations are indicated for the severe complicated cases which could not be cured by tear-closing diathermy alone. During the early period, almost all the authors applied lamellar scleral resection, which is a sort of eye-shortening operation, to cases with a very poor prognosis. Later, numerous modifications of technique of the eye-shortening operations were published, all of which, however, originated from the idea of lamellar scleral resection; and it seems that this procedure came to have somewhat different indications for each author, being considerably limited by some, and very wide for others, such as Swan and Malbrán.

At the beginning of our performance (from 1951 to 1956), our personal indications for lamellar scleral resection were strictly limited to cases, where (1) diathermy alone was not considered effective because of the extreme severity, (2) tear-closing diathermy had been used in vain, or had even made matters worse.

But with the accmulation of experience the following condition has lately been added as an indication: (3) cases where the use of lamellar scleral resection might benefit by closing retinal tears, or keeping the retina and choroid in contact.

Consequently the use of this procedure has increased considerably in its frequency since 1957. In the 68 eyes treated by lamellar scleral resection during the period of this statistical investigation (January 1953 to June 1959), the clinical conditions which necessitated this procedure could be classified as follows:

A. Those operated on primarily.

1. Those with either giant tears or many tears covering a wide area of the fundus, (those with slight or no detachment were excluded).
2. Those with total detachment and tear.
3. Those with partial detachment of long standing with a tear showing no flattening on preoperative rest.
4. Those in which no tear could be detected.
5. Those with aphakia (either with or without a tear)

B. Those operated on secondarily following unsuccessful diathermy.

6. Those with a remaining flat detachment in a part of the fundus.
7. Those with an insufficiently closed or newly developed tear, still showing no improvement or even aggravation of the detachment.
8. Those with a cicatrized tear, but with no improvement or even aggravation.
9. Those with no tear showing no improvement following blind operation.
10. Those with aphakia.

Results

Among the 68 eyes treated with lamellar scleral resection, two should be excluded from the present investigation, because the final results are not yet known. In the remaining 66 eyes, the results are summarized:

A. Primary operations.

1. Those with either a giant tear or many tears (9 eyes):

Complete reattachment was obtained in 6 eyes (66.7%), improvement in one, and failure in two.

2. Those of total detachment with a tear (5 eyes):

Complete reattachment occurred in only one eye, improvement in one, and failure in three.

3. Those of partial detachment of long standing with a tear showing no flattening on preoperative rest (3 eyes):

Complete reattachment in 2 eyes, improvement in one, and no failure.

4. Those in which no tear could be detected (10 eyes):

Complete reattachment in only one eye, improvement in two, and failure in 7.

5. Those with aphakia (7 eyes, with or withour a detectable tear):

Complete reattachment was obtained in two eyes (28.6%), improvement in two, and failure in three.

B. Secondary operation.

6. Those with a remaining flat detachment in a part of the fundus:

In the majority of the eyes belonging to this group, a cicatricial closure of the tear was incomplete following diathermy. In such cases we considered it beneficial to apply lamellar scleral resection in order to close the tear as well as to keep the retina and choroid in as close contact as possible. Among these 5 eyes, complete reattachment was obtained in 4 eyes (80%), improvement in one, and no failure.

7. Those with insufficiently closed or newly developed tears, still showing no improvement or even aggravation of the detachment (11 eyes):

Complete reattachment occurred in only two eyes (18.2%), improvement in one, and failure in eight.

8. Those with cicatrized tears, but no improvement or even aggravation of the detachment (10 eyes):

Complete reattachment in two eyes(20%), improvement in one, and failure in seven.

9. Those with no tear:

In two eyes, which showed no improvement following blind diathermy operation, complete reattachment was obtained in one eye, and failure in the other.

10. Those with aphakia:

Diathermy operation was used first because of the presence of a relatively small tear, but no improvement was obtained. Among these 4 eyes, complete reattachment was obtained in two eyes, and failure in two.

Table 2. Results of Operation for Retinal Detachment

	A. Scleral Resection				B. Tear-Closing Diathermy Operation				C. Total			
	Number of Eyes	Complete Reattachment	Improved	Failure	Number of Eyes	Complete Reattachment	Improved	Failure	Number of Eyes	Complete Reattachment	Improved	Failure
1953	2	0	1	1(50%)	44	34(77.3%)	2	8(18.2%)	46	34(73.9%)	3	9(19.6%)
1954	4	1(25%)		3(75%)	63	51(81.0%)	1	11(17.5%)	67	52(77.6%)	1	14(20.9%)
1955	4	0		4(100%)	67	47(70.1%)	4	16(23.9%)	71	47(66.2%)	4	20(28.2%)
1956	5	4(80%)		1(20%)	60	45(75%)	8	7(11.7%)	65	49(75.4%)	8	8(12.3%)
1957	18	4(22.2%)	6	8(44.4%)	53	44(83%)	4	5(9.4%)	71	48(67.6%)	10	13(18.3%)
1958	20	9(45%)	1	10(50%)	72	62(86.1%)	6	4(6.5%)	92	71(77.2%)	7	14(15.2%)
1059 Jan. th June	13	5(38.5%)	2	6(46.2%)	26	22(84.6%)	2	2(7.7%)	39	27(69.2%)	4	8(20.5%)
Total	66	23(34.8%)	10	33(50%)	385	305(79.2%)	27	53(13.8%)	451	328(72.7%)	37	86(19.1%)

Comment

Our personal results are shown in Table 2. In 66 eyes subjected to this procedure complete reattachment was obtained in 23 eyes, 34.8% (Table 2A). This incidence of operative success was not as good as was reported recently by American or European authors. Diathermy operation not combined with scleral resection, showed a considerably high incidence of success: 79.2% (Table 2B). Further, the therapeutic records as a whole (Table 2C) showed 72.7% success, which is almost identical with the reports of numerous authors.

The relatively poor incidence of success of lamellar scleral resection seems to be due to the fact that our indications are still more strict than those of other authors, and we choose only severe, complicated cases, although our indications have been gradually widened for the past several years. Recently (1958, 1959), with this operation being more widely indicated, the incidence of success has become higher (45%, 38.5% respectively) than the total average (34.8%) (Table 2A), and it can be expected that the wider indications become, the higher the incidence of success will be.

As shown in Table 2C, the total incidence of success was almost the same in years with many cases given the scleral resection and in those with few. Table 3 shows, however, 34.4% of complete reattachment in 32 eyes treated with scleral resection secondarily after the failure of a preceding diathermy

Table 3. Results of Lamellar Scleral Resection Performed Primarily and Secondarily

	Number of Eyes	Complete Reattachment	Improved	Failure
Those Operated on Primarily	34	12(35.3%)	7	15(44.1%)
Those Operated on Secondarily	32	11(34.4%)	3	18(56.3%)
Total	66	23(34.8%)	10	33(50.0%)

operation. Therefore, it must be pointed out that lamellar scleral resection is of considerable significance in the area of retinal-detachment surgery.

Summary

During the period from January 1953 to June 1959, 453 eyes with retinal detachment were treated surgically. Of these 68 eyes (15%) underwent lamellar scleral resection primarily or secondarily. With the exclusion of two eyes in which the final results are not yet known, complete reattachment of the retina was obtained in 23 eyes (34.8%). A somewhat poor incidence of success is due to the fact that only complicated severe cases with a poor prognosis were treated with scleral resection. On the other hand, in the remaining 385 eyes which were operated on by diathermy alone success was obtained in 305 eyes (79.2%).

Our personal indications up to date are described.

(Published in J. of Clinical Ophthalmology 14:651, 1960 in Japanese).

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EXTRACTION AND FIBRIL RECONSTITUTION OF CORNEAL COLLAGEN

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Collagen fibrils are the major component of the corneal stroma and play an important role in the maintenance of corneal transparency, wound healing, swelling and so on.

Although there have been many morphological studies on the corneal collagen, very little was known about the biochemistry and physical-chemistry of corneal collagen because of the difficulties of extraction in its native state (not as the gelatin).

Nageotte found the fibrous precipitate from the extract of rat-tail-tendon stained similarly to collagen fibres in tissue[1], and from the study of X-ray diffraction[2] and electron microscopy[3,4] this fibrous precipitate proved to have the same periodicity as collagen fibrils in tissues. It was called reconstituted collagen fibrils. Hitherto, extraction of soluble collagen from diverse tissues and the reconstitution of collagen fibrils has been studied by many investigators.

Under the electron microscope collagen fibrils show a periodicity of 650 Å, and it is this characteristic of the collagen fibril, that distinguishes it from other fibrils. In the cornea too, collagen fibrils show this periodicity, though the periodicity is not so clear *in situ*[5].

Extraction and fibril reconstitution of corneal collagen has not been studied previously.

This paper describes extraction of native corneal collagen (acid soluble and insoluble) and the *in vitro* reconstitution of collagen fibrils from these extracts.

Details of this work have already been presented in three preliminary communications (in Japanese)[6,7,8].

Experimental

1. Material.

The corneas used were fresh beef and rat corneas. The epithelium had been scraped away beforehand. The corneas were cut into pieces with scissors, soaked in several changes of 50 volumes of cold 10% sodium chloride with stirring, then washed thoroughly with several changes of distilled water. In these procedures the corneas showed marked swelling, reaching 15 to 20 mm in thickness.

2. Extraction of acid soluble collagen.

The tissue was suspended in 2 volumes of 0.001 M acetic acid or 0.01 M citric acid and allowed to stand in the cold for 3 to 90 days. The extract was clear and not very viscous.

3. Extraction of insoluble collagen.

The residue from extraction of acid soluble collagen was incubated at 25°C for 90 hours in the presence of 0.2% pepsin in 0.5% acetic acid. The bulk of the cornea was digested by this method, yielding a very viscous and highly opalescent extract.

4. Purification of extracts.

The swollen mass was squeezed through bolting cloth and the extract was filtered successively through coarse, medium and fine glass filters with suction in the cold.

In the case of extracted insoluble collagen the filtrate was dialysed against 0.02 M dibasic sodium phosphate and the precipitates were collected and repeatedly washed in dibasic sodium phosphate and distilled water successively, and then redissolved in 0.05% acetic acid or 0.15 M citric buffer pH 3.8.

5. *In vitro* reconstitution of collagen fibrils.

5a. Normally spaced fibrils. Collagen was precipitated as a fibrous mass by addition of salt or mucopolysaccharides (0.1% NaCl or a low concentration of chondroitin sulphate, keratosulphate and heparin), by pH adjustment (for instance, addition of citric buffer, to a final concentration of 0.5 M pH 4.9) and upon dialysis (against 0.02 M dibasic sodium phosphate, 0.5 M citric buffer pH 4.9, distilled water and so on).

Acid soluble collagen was so low in concentration, that the precipitate was small in quantity and barely seen with the naked eye. On the contrary, extracted insoluble collagen yielded a massive fibrous precipitate.

Electron microscope observations of these precipitates were made with a Hitachi HU 10 Electron Microscope with chromium shadowing, and were identified as reconstituted collagen fibrils having characteristic cross striations with the periodicity of 400 to 900 Å, average 675±41 Å (Fig. 1). The fibrils had tapered and spindle shaped ends and each period was further divided into three sub-bands. (Fig. 6–Fig. 11)

It was more difficult to obtain uniformly developed fibrils with cross striations from extracted insoluble collagen, than from acid soluble collagen. Fibril breadth was determined by various precipitating conditions, lapse of time, pH, ionic strength, temperature and so on. Table 1 indicates the breadth of the fibrils reconstituted

Fig. 1. Periodicity of normally spaced fibrils.

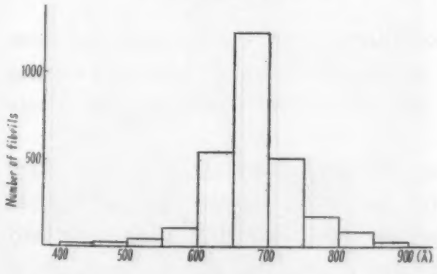


Table 1.

Fibril breadth	Number
0～ 100Å	2500
101～ 200Å	912
201～ 400Å	498
401～ 800Å	108
801～1600Å	75
1601～2400Å	36
2401～3200Å	6

Fig. 2. Segment length of FLS. (chondroitin sulphate)

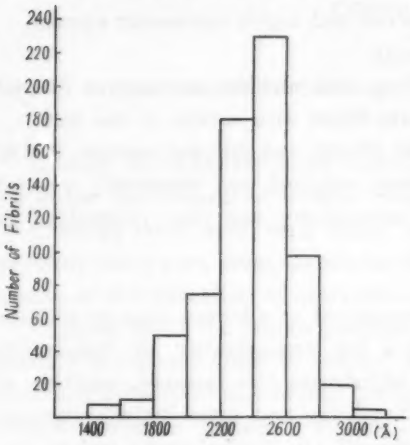


Fig. 4. Segment length of SLS.

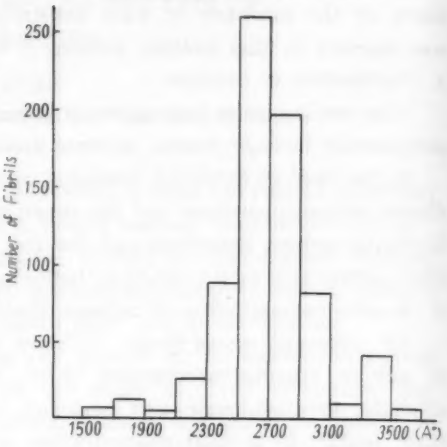


Fig. 3. Segment length of FLS (heparin).

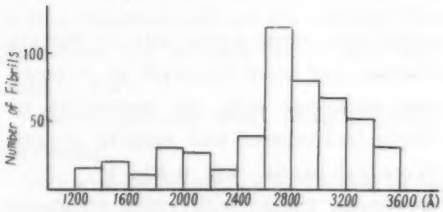
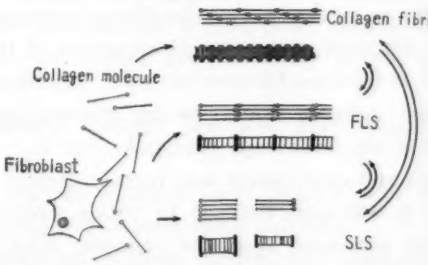


Fig. 5. (from H. Noda. The Journal of Japanese Biochemical Society 31:664. 1959)



from 0.5 mg/ml extracted insoluble collagen dialysed aga inst 0.5 M citric buffer pH 4.9 for 24 hours at 0°C.

5b. Fibrous long spacing (FLS). Mucopolysaccharides such as chondroitin sulphate, keratosulphate and heparin were added to the collagen solution with the concentration of ca. 10 mg/ml.

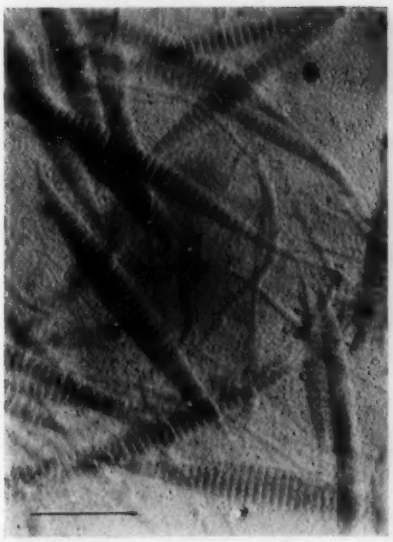
The fibrous long spacing (FLS) were reconstituted with fine symmetrical cross striations (Fig. 12-Fig 15). Their segment length was 1400 to 3200 Å, averaging 2350±177 Å with chondroitin sulphate (Fig 2), and 1200 to 3600 Å averaging 2640 Å with heparin (Fig. 3).

If on adding mucopolysaccharides to the collagen solution sodium acetate is mixed simultaneously, pH is adjusted to 5 to 7, and the mixture is dialysed against distilled water immediately after mixing, then uniformely developed FLS were constantly obtained. Without dialysis and pH adjustment, the precipitated fibrils often failed to show cross striations and rarely took the form of FLS, indicating, that the ionic

Normally spaced fibrils from corneal acid soluble collagen.

Fig. 6.

Fig. 7.



Scale 10, 000 Å (×15. 500)

Normally spaced fibrils from corneal extracted insoluble collagen.

Fig. 8.

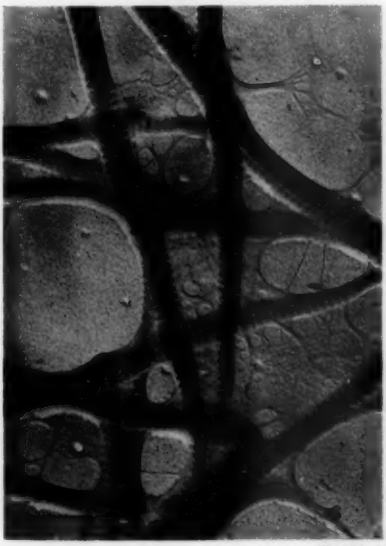
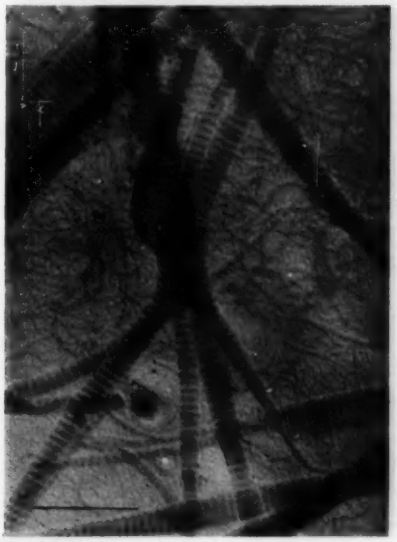


Fig. 9.



Scale 10, 000 Å (×15, 500)

Fig. 10. Normally spaced fibrils from corneal extracted insoluble collagen. (Latex, 880Å.)

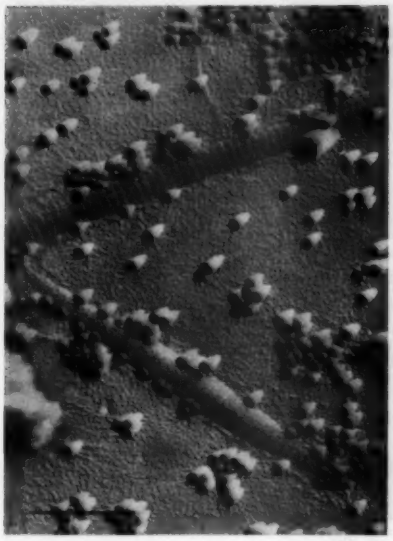
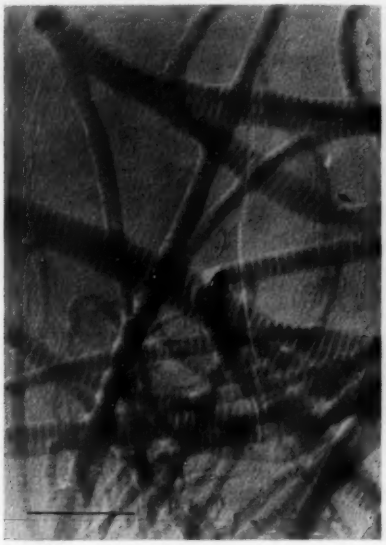


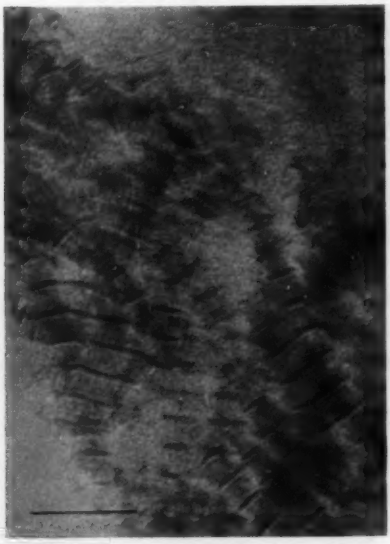
Fig. 11. Normally spced fibrils precipitated with keratosulphate.



Scale 10, 000 Å (×15, 500)

Fig. 12. Fibrous long spacing precipitated with chondroitin sulphate from corneal acid soluble collagen.

Fig. 13. Fibrous long spacing precipitated with chondroitin sulphate from corneal extracted insoluble collgen.



Scale 10, 000 Å (×15,500)

Fig. 14. Fibrous long spacing precipitated with heparin. (Latex, 83)Å.)

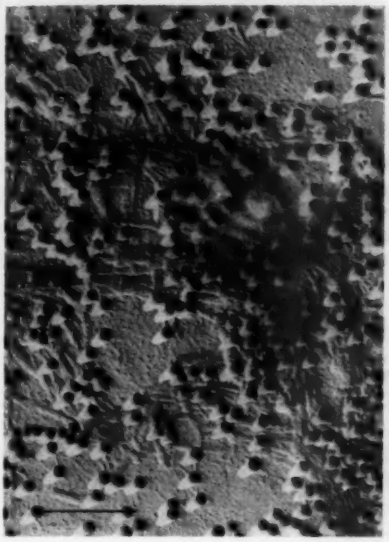


Fig. 15. Fibrous long spacing precipitated with heparin.

Scale 10, 000 Å (×15, 500)

Segment long spacing from acid soluble collagen.

Fig. 16.

Fig. 17.

Scale 10, 000 Å (×21, 500)

Segment long spacing from extracted insoluble collagen.

Fig. 18.

Fig. 19.

Scale 10, 000 Å (×21, 500)

strength played some role in the precipitation of FLS.

5c. Segment long spacing. Adenosine triphosphate (made natrium free by passing through a column of Amberlite IR 120 acid type) was added to the collagen solution (in 0.05% acetic acid) in the cold with the concentration ratio of 1 mg adenosine triphosphate to 1 mg collagen (final pH 2.2). Long spaced fibrils, one segment in length (SLS) with asymmetrical fine cross striations were precipitated. (Fig. 16-Fig. 19). Their segment length was 1500 to 3700 Å, average 2690±231 Å (Fig. 4). It took 4 hours for SLS to aggregate when acid soluble collagen was used, and 24 hours, when extracted insoluble collagen was used. SLS became broader as time elapsed.

Discussion

Reconstituted collagen fibrils with normal spacing from the cornea showed typical cross striations with the periodicity of 675±41 Å, the same as collagen fibrils *in situ* and reconstituted collagen fibrils obtained from other tissues. Corneal collagen fibrils *in situ* showed a uniform breadth of 300 Å and did not have tapered ends, differing from the reconstituted fibril only at this point. This fibril reconstitution verifies that the extracted collagen was in the native and not the denatured state.

Younger rats had a relatively large amount of soluble collagen in the cornea, as had been the case in the skin[10], and this gradually gave place to insoluble collagen as they grew older. At any rate, as compared with other tissues, the cornea had very small quantities of acid soluble collagen, as Polatnick et al. had pointed out[11].

Although it is of great importance to study the insoluble collagen, the leading

component of corneal collagen, nothing is known about its physico-chemical properties because of its insolubility.

Insoluble collagen was almost the same as soluble collagen in the results of the X-ray diffraction, electron microscopy, amino acid analysis and so on. The differentiating features between insoluble and soluble collagen is open to question.

Collagen is highly resistant to enzymatic digestion in the native form. It is hydrolysed only by collagenase with a high degree of substrate specifity. Supposing that the insoluble collagen fibril was made from a certain linkage of soluble collagen molecules, this linkage would be cut by enzymes not active on the soluble collagen molecule, and thus insoluble collagen could be extracted in the native state.

Pepsin, trypsin and hyaluronidase were used under this supposition and pepsin proved to be most effective in the extraction and solubilization of the corneal insoluble collagen.

As a matter of course, with lower pH and higher temperature, the extraction was more drastic, and it was not so difficult to extract the whole insoluble collagen and liquefy the cornea. In this case, care must be taken not to denature the collagen, for, the lower the pH, the lower the denaturation temperature.

Fibril reconstitution from the extracted insoluble collagen indicated, that the insoluble collagen was not denatured and was in the native state, though the precipitating conditions were slightly different from those required for acid soluble collagen, suggesting slight modification on molecular properties.

FLS and SLS, first found by Schmitt et al[12,13], were not found *in situ*, and thought to be artificial products, were precipitated from corneal collagen by the addition of mucopolysaccharides in the former and adenosine triphosphate in the latter case.

Mucopolysaccharides have been thought to play an important role in fibrillogenesis *in vivo*, and precipitation of FLS by them suggests that they in fact play some role in corneal fibrillogenesis *in vivo*. The fact that the amount of mucopolysaccharides used for the precipitation of FLS was larger as compared with the physiologic concentration of mucopolysaccharides in the cornea opposed this suggestion, and the fact that low concentrations of mucopolysaccharides added to the collagen solution yielded a normally spaced fibril supported it.

Reconstitution of SLS by the addition of an adequate amount of adenosine triphosphate, the key material in the biological energy cycle, and reconstitution of fibrils without spacing by low concentrations, suggested the possibility of adenosine triphosphate playing some role in fibrillogenesis *in vivo*.

Normally spaced fibrils, FLS and SLS were interconvertible in the collagen solution obtained from the carp swimm bladder, indicating that each of them was composed of the same collagen molecule[13].

Schmitt et al. assumed, that the collagen molecule was an asymmetric, rod-like macromolecule having a length of 2000 Å to 3000 Å, and illustrated the formation of the three types of fibrils by the mechanisms shown in Fig. 5[14]. If the collagen molecules lay parallel laterally in identical orientation, they formed SLS with the length of 2000 to 3000 Å having fine asymmetric cross striations, and if they were arranged parallel laterally with reciprocal direction, they formed FLS with the periodicity of 2000 to 3000 Å having symmetrical fine cross striations. Normal spacing was the case, when the collagen molecules were arranged longitudinally with a slight phase discrepancy to the adjacent arrangements making fine asymmetrical cross striations, visible only by phospho-tungustic acid staining.

The molecule of corneal collagen had a length of 3000 to 3500 Å measured by flow-birefringence, supporting this hypothesis. (Details of the physico-chemical properties of the corneal collagen will be published elsewhere.)

Collagen molecules seemed to have the tendency to aggregate easily and thus it is not appropriate to single out one factor from many (salt concentrations, pH, ionic strength, temperature, mucopolysaccharides, adenosine triphosphate and so on) as the only factor responsible for fibrillogenesis *in vitro* and *in vitro*.

Summary

1. Acid soluble collagen was extracted from rat and beef corneas in the native state with dilute acid.

2. Insoluble collagen was extracted in the native state by treating the cornea with pepsin at 25°C for 90 hours.

3. *In vitro* reconstituted fibrils from acid soluble and extracted insoluble collagen were observed with the electron microscope.

a. Normally spaced fibrils were precipitated by the addition of salt or small amounts of mucopolysaccharides, by pH adjustment and upon dialysis. Their periodicity fell within the range of 400 to 900 Å, average 675±41 Å, and this period was further divided into three sub-bands, the similar to the collagen fibril *in situ* and the reconstituted collagen fibrils obtained from other tissues.

b. Fibrous long spacing (FLS) were precipitated by the addition of mucopolysaccharides such as chondroitin sulphate, keratosulphate and heparin. The segment length was 1400 to 3200 Å, average 2350±177 Å with chondroitin sulphate and 1200 to 3600 Å, average 2640 Å with heparin.

c. Segment long spacing (SLS) were precipitated by the addition of adenosine triphosphate. The segment length was 1500 to 3700 Å, average 2690±231 Å.

4. Mechanisms of *in vitro* reconstitution of these three types of fibrils and fibrillogenesis *in vivo* are discussed.

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STUDIES OF THE FIXATION MOVEMENT OF THE EYE BALL

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Many ways to observe the eye movement had been devised since Johannes Müller in the 19th century. A photographic method for its purpose was first adopted by Stratton, while a cinematographic one was by Judd both in the early stage of this century.

The author, being ocuppied in the management of the patients with amblyopia ex anopsia, was much interested in the real state of their fixation and succeeded in creating a new optical method for observing, recording and analyzing the fixation movement of the eye ball, in which a newly planned converging light producing apparatus, a very small planesmirror attached to the corneal limbus, 16 mm. motion picture camera with a specially designed pint-glass in front, etc. were employed.

The detailed plan of the new converging light producing apparatus is given in Fig. 1 and the arrangement of each part (converging light producing apparatus, subject, lamp-light for fixation, cine-camera etc.) is illustrated in the Fig. 2.

My new apparatus makes its round image at the place 144 cm. distant from the part of the pin-hole and its size is only about 0.5 mm. in diameter, when the smaller pin-hole (0.1 mm.) is used. Therefore, a mirror being inserted in the pathway of the converging light bundle, its reflected image is to be made at the same distance.

Very small plane mirror ($0.5 \times 0.8\ mm^2$, 0.08 g.) is attached to the lateral corneal limbus of the subject, who is watching a small lamp-light for fixation in the dark room. Then, each part is so arranged, that the converging light bundle is reflected

Fig. 1.

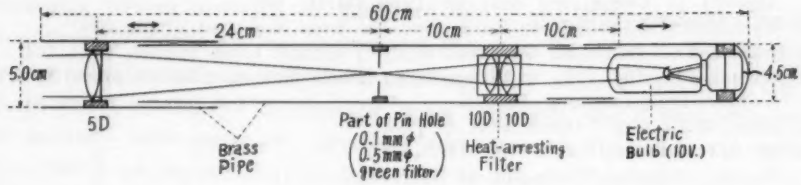


Fig. 2.

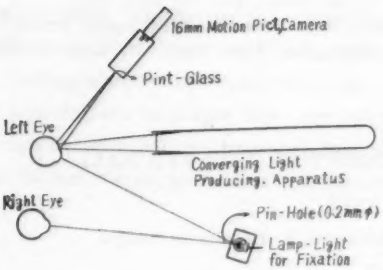
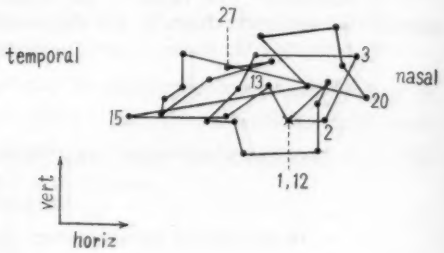


Fig. 3.



with the mirror and throws its focalized image as a sharp point on the pint-glass $(9\times11\,\mathrm{cm})^2$ which is just in front of the camera, Cine Kodak Special.

Now, the experimenter, watching the movement of the point on the pint-glass, can not only observe a big magnified micro-movement of the eye ball at fixation, but also record it simultaneously on the film (Fuji's Super Panchromatic Negatype Film ASA 100) in the Cine Kodak Special. The number of film rotation being 12 pieces per second, the time of exposure to each piece is about 1/23 second.

Fig. 3 illustrates several pieces of the actual cine-film, on which the focalized images are shown as small dots.

For the purpose of reappearing and analizing the fixation movement given now on the film, the author projected the film piece by piece on a section-paper and each site of the dot was marked on it. Thus, a series of dots appears on the paper. This is the reappearance of the fixation movement of the eye ball in diagram and may at the same time, be thought to be representing the movement of the site of fixation on the retina.

For its detailed analysis, further calculation is required. When a mirror fixed on the corneal limbus moves by α degree, the reflected ray with the mirror moves by $2\,\alpha$ degree.

Then, an eqation can be written as below approximately:

$$\alpha = \frac{90}{\pi D} d$$

where

α = the amount of the micro-movement of the eye ball in degree

π = the ratio of the circumference of a circle to its diameter

D = the distance between the mirror (eye ball) and the pint-glass (in the case of normal eye, 500 mm and in the case of amblyopic eye, 200~300 mm.)

d = the distance between a point (focalized image) and the successive one on the pint-glass

$D >>> d$

Study of the Physiological Fixation Movement

(Especially a comparative study on steadiness of the binocular fixation with that of monocular one)

The author, at first, investigated the fixation movement of 10 normal adults espcially with regard to the difference between the steadiness of the binocular fixation and the monocular one, of which only a few reports are found in the literature,

The followings are example diagrams obtained in cases of a female adult of 21 years of age.

Fig. 4 illustrates the state of binocular fixation, while Fig. 5 shows that of the monocular one (right eye). In these diagrams, the movements are shown enlarged as big as 4 times the original ones on the pint-glass. The figures marked on the diagrams show the order of movement of the points

Fig. 4.

Fi8. 5.

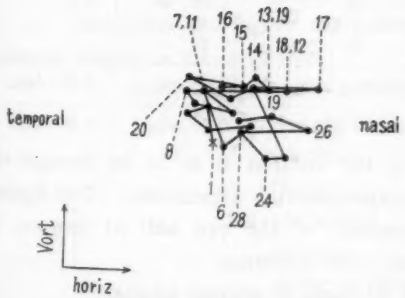
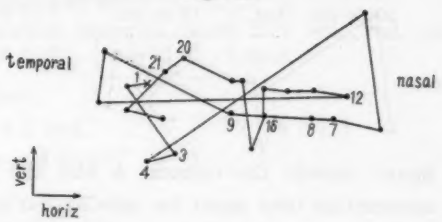


Fig. 6.



Tab. 1 gives further explanations on the diagrams above.

The figures in the column A are the order of movement. The figure outside the column A is the time of experiment. In the column B, the time of fixation at each point (so-called elemental fixation) is shown in second. The difference between the

Tab. 1. Case 1. (21 years. ♀ old.) v.d.=0.7 (1.5×—0.5 D.) v.s.=0.1 (1.5×—0.5 D.)

Binocular Fixation				Monocular Fixation			
A		B	C	A		B	C
1		0.71 sec.	0.18 deg.	1		0.71 sec.	0.08 deg.
2		0.88 〃	0.07 〃	2		0.13 〃	0.26 〃
3		0.38 〃	0.07 〃	3		0.38 〃	0.04 〃
4		0.63 〃	0.17 〃	4		0.38 〃	0.17 〃
5		0.38 〃	0.07 〃	5		0.46 〃	0.19 〃
6		0.54 〃	0.12 〃	6		0.54 〃	0.28 〃
7		0.71 〃	0.17 〃	7		0.38 〃	0.15 〃
8		0.54 〃	0.17 〃	8		1.29 〃	0.14 〃
9		0.38 〃	0.15 〃	9		0.29 〃	0.08 〃
10		2.04 〃	0.19 〃	10		0.63 〃	0.13 〃
11		0.38 〃	0.14 〃	11		0.29 〃	0.12 〃
12		0.29 〃	0.44 〃	12		0.90 〃	0.12 〃
13		0.71 〃	0.13 〃	13		0.54 〃	0.14 〃
14		0.54 〃	0.19 〃	14		1.38 〃	0.12 〃
15		1.21 〃	0.06 〃	15		1.21 〃	0.32 〃
16		1.88 〃	0.10 〃	16		0.71 〃	0.14 〃
17		1.29 〃	0.14 〃	17		0.38 〃	0.18 〃
18		0.71 〃	0.15 〃	18		1.04 〃	0.34 〃
19		0.21 〃	0.13 〃	19		0.54 〃	0.27 〃
20		1.71 〃	0.34 〃	20		0.88 〃	0.21 〃
21		0.63 〃	0.57 〃	21		1.21 〃	0.29 〃
22		0.21 〃	0.38 〃	22		1.63 〃	0.07 〃
23		0.96 〃	0.07 〃	23		0.29 〃	0.32 〃
24		0.54 〃	0.13 〃	24		0.29 〃	0.22 〃
25		0.79 〃		25		0.96 〃	0.40 〃
20.25 sec.	Tot.	19.25 sec.	4.33 deg.	26		1.29 〃	0.42 〃
	Aver.	0.77 sec.	0.18 deg.	27		0.29 〃	
				20.08.sec.	Tot.	19.02 sec.	5.20 deg.
					Aver.	0.70 sec.	0.20 deg.

figure outside the column A and the total in the column B is to be thought the summed-up time spent for moving during the corressponding experiment. The figures in the column C show the amount of the movement of the eye ball at fixation in degree calculated according to the formerly mentioned equation.

Tab. 2 is the summarization of the data in 10 cases of normal adults.

The conclusion of this study is the following;

1) The course of the fixation movement of an eye ball is quite irregular like Brown's movement.

2) The time of the so-called elemental fixation is 0.49~0.87 sec. on an averge.

Tab 2.

	Time of Elemental Fixation (aver. sec.)		Interval of bigger Movement (aver. sec.)		Amount of Micro- movement in sec. (deg/sec.)	
	Binocular F.	Monocular F.	Binocular F.	Monocular F.	Binocular F.	Monocular F.
Case 1 (21, ♀) v.d=0.7 (1.5×—0.5) v.s=0.1 (1.5×—0.5)	0.77	0.70	0.85	0.77	0.21	0.26
Case 2 (21, ♀) v.d=0.7 (1.2×—1.0) v.s=0.7 (1.5×—1.5)	0.65	0.87	0.72	0.95	0.22	0.16
Case 3 (21, ♀) v.d=0.4 (1.0×—1.5) v.s=0.1 (1.0×—1.0)	0.74	0.49	0.77	0.55	0.20	0.34
Case 4 (22, ♂) v.d=1.2 v.s=1.5	0.67	0.86	0.73	0.93	0.36	0.36
Case 5 (21, ♀) v.d=1.2 v.s=1.0	0.57	0.61	0.63	0.67	0.52	0.37
Case 6 (21, ♀) v.d=1.5 v.s=1.5	0.51	0.49	0.57	0.55	0.31	0.29
Case 7 (21, ♀) v.d=1.5 v.s=1.5	0.77	0.75	0.86	0.83	0.41	0.38
Case 8 (21, ♂) v.d=0.4 (0.8×—1.0) v.s=0.7 (0.7×—0.5)	0.59	0.57	0.66	0.63	0.37	0.40
Case 9 (22, ♂) v.d=1.5 v.s=1.5	0.87	0.55	0.95	0.61	0.26	0.46
Case 10 (26, ♂) v.d=1.5 v.s=1.5	0.49	0.56	0.55	0.63	0.23	0.32
aver.	0.66	0.65	0.73	0.71	0.31	0.33

Enumerating the reports up to this time,

accrding to Öhrwall 1~1.5 sec

Adler & Fliegerman 1 sec.

Okuse 0.58~2.61 sec.

3) The quick and bigger movement at fixation happens every 0.71 sec. and its average degree is 9.4~30.9 min.

according to Marx & Trendelenburg

horizontal 3~8.5 min.

vertical 5.6~7.6 min.

Okuse

horizontal 7.5~17.3 min.

vertical 5.4~15.5 min.

Adler & Fliegerman

12 min. 29 sec.~17 min. 31 sec.

Dohlman 45~50 min.

4) Statistically (calculated according to the method of stochastics) there is little difference between the steadiness of binocular fixation and that of the monocular one.

Fig. 7.

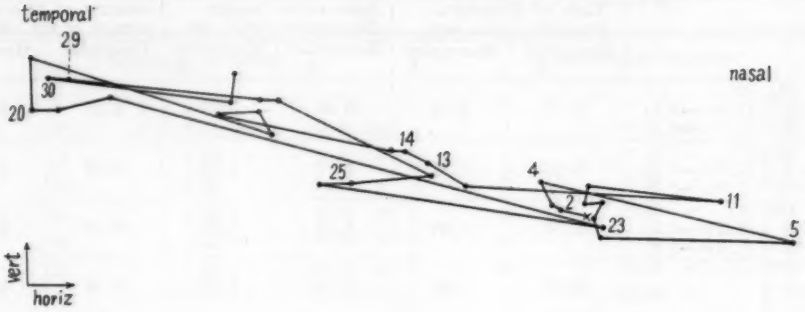
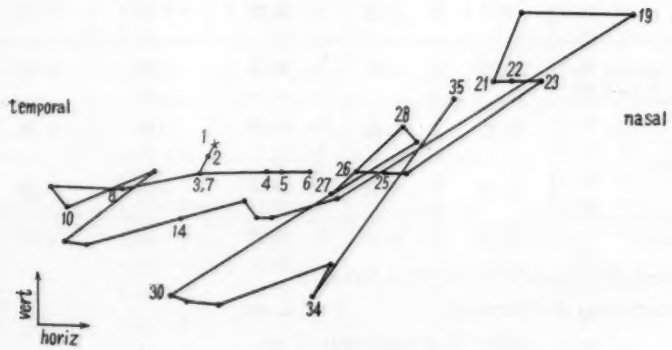


Fig. 8.



Study of the Fixation Movement in Amblyopia ex Anopsia

The author then investigated the micro-movement of the eye ball in 13 cases with amblyopia ex anopsia (parafoveal fixation 5, paramacular f. 4 and peripheral f. 4).

For instance, Fig. 6 is one of the diagrams showing the state of fixation in cases with parafoveal fixation and Tab. 3 shows the details corresponding to Fig. 6.

In these diagrams, the fixation movement is shown enlarged 2 time as big as the original, seen on the pint-glass.

The figures in the column A and B have the same meaning with those in the previous chapter. The figures in the column C this time are obtained supposing, that an eye ball rotates around the centre of rotation. If so, an equation is given as the following;

$$x \fallingdotseq \frac{11}{D+13} d$$

Tab. 3. Strabismus convergens
(17 years old, δ v.d. (cortical)=0.1
parafoveal Fixation)

A	B	C
1	0.38 sec.	0.13 mm.
2	0.04 〃	0.09 〃
3	0.04 〃	0.10 〃
4	0.21 〃	0.18 〃
5	0.71 〃	0.13 〃
6	0.21 〃	0.23 〃
7	0.29 〃	0.08 〃
8	0.21 〃	0.06 〃
9	0.96 〃	0.15 〃
10	0.13 〃	0.14 〃
11	0.13 〃	0.32 〃
12	0.04 〃	0.06 〃
13	0.04 〃	0.08 〃
14	0.04 〃	0.06 〃
15	0.13 〃	0.08 〃
16	0.54 〃	0.37 〃
17	0.04 〃	0.12 〃
18	0.21 〃	0.08 〃
19	0.71 〃	0.31 〃
20	0.21 〃	0.18 〃
21	0.29 〃	0.19 〃
22	0.63 〃	0.30 〃
23	0.13 〃	0.06 〃
24	0.04 〃	0.15 〃
25	0.04 〃	0.26 〃
26	0.04 〃	0.14 〃
27	0.13 〃	0.13 〃
28	0.63 〃	
8.33 sec.	Tot. 7.20 sec.	4.18 mm.
	Aver. 0.26 sec.	0.15 mm.

Tab. 4. Strabismus sursum-convergens
(17 years old, δ v.s (cortical)=0.1
paramacular Fixation)

A	B	C
1	0.04 sec.	0.09 mm.
2	0.04 〃	0.10 〃
3	0.46 〃	0.39 〃
4	0.13 〃	0.09 〃
5	0.13 〃	0.15 〃
6	0.38 〃	0.63 〃
7	0.04 〃	0.44 〃
8	0.04 〃	0.40 〃
9	0.21 〃	0.14 〃
10	0.13 〃	0.53 〃
11	0.21 〃	0.63 〃
12	0.29 〃	0.12 〃
13	0.21 〃	0.53 〃
14	0.38 〃	0.39 〃
15	0.04 〃	0.12 〃
16	0.29 〃	0.08 〃
17	0.38 〃	0.39 〃
18	0.54 〃	1.96 〃
19	0.04 〃	0.63 〃
20	0.13 〃	0.41 〃
21	0.46 〃	0.10 〃
22	0.21 〃	0.17 〃
23	0.21 〃	0.93 〃
24	0.13 〃	0.13 〃
25	0.13 〃	0.13 〃
26	0.21 〃	0.18 〃
27	0.04 〃	0.55 〃
28	0.21 〃	0.12 〃
29	0.13 〃	1.65 〃
30	0.13 〃	0.10 〃
31	0.54 〃	0.17 〃
32	0.21 〃	0.71 〃
33	0.38 〃	0.22 〃
34	0.29 〃	
8.75 sec.	Tot. 7.39 sec.	13.38 mm.
	Aver. 0.22 sec.	0.41 mm.

Tab. 5. Strabismus convergens
(23 years old, ♀ v.d (cortical)=0.01,
without Fixation.)

A	B	C
1	0.04 sec.	0.13 mm.
2	0.04 〃	0.08 〃
3	0.13 〃	0.12 〃
4	0.21 〃	1.38 〃
5	0.21 〃	1.02 〃
6	0.04 〃	0.12 〃
7	0.04 〃	0.07 〃
8	0.21 〃	0.07 〃
9	0.13 〃	0.09 〃
10	0.21 〃	0.68 〃
11	0.04 〃	1.33 〃
12	0.04 〃	0.24 〃
13	0.04 〃	0.13 〃
14	0.13 〃	0.07 〃
15	0.13 〃	0.93 〃
16	0.13 〃	0.20 〃
17	0.29 〃	0.14 〃
18	0.21 〃	1.32 〃
19	0.46 〃	0.29 〃
20	0.63 〃	0.13 〃
21	0.04 〃	0.29 〃
22	0.13 〃	2.67 〃
23	0.46 〃	1.50 〃
24	0.54 〃	0.15 〃
25	0.29 〃	0.44 〃
26	0.38 〃	0.90 〃
27	0.04 〃	1.21 〃
28	0.46 〃	0.10 〃
29	0.04 〃	0.87 〃
30	0.04 〃	0.11 〃
31	0.04 〃	
7.08 sec.	Tot. 5.82 sec.	16.78 mm.
	Aver. 0.19 sec.	0.56 mm.

where

x=the amount of change in the site of fixation on the retina in mm.

D=the distance between the mirror (eye ball) and the pint-glass (in this case, 200~300 mm.)

d=the distance between a point (focalized image) and the successive one on the pint-glass

D>>d

Strictly speaking, the figure x thus calculated is not so precise, but still deserves such a role as a standard to each other in the problem of steadiness of fixation of the amblyopic eyes.

The conclusion of this study:

1) According to Kestenbaum, the state of fixation of the amblyopic eye is called Fixationsnystagmus. However, it is not always like nystagmus and is quite irregular and unsteady.

2) Steadiness of fixation in amblyopia is closely related to the site of fixation on the retina. The further the site is from the true fovea, the more unsteady fixation becomes.

Summary

The author successfully created a new optical method for observing, recording and analyzing the fixation movement of the eye ball, in which a newly planned converging light producing apparatus, a very small plane mirror attached to the corneal limbus, 16 mm. motion picture camera with a specially designed pint-glass in front etc, were employed.

Then, the details of the fixation movements of the eye balls both in cases of normal adults and the patients with amblyopia ex anopsia were studied, proving this method to be of value.

Tab. 6.
(The Tab. 6 is the summarization of the data in 13 cases with amblyopia.)

Case.	Age.	Sex.	Strabismus	Visual Acuity of Squinting Eye (cortical)	Refractive Error	Site of Fixation	Fixation Time (sec.)	Amount of change in the site of Fixation on the Retina (mm/sec)
Control				normal		foveal	0.49~0.87	0.03~0.14
1	17	♂	convergens	0.1	+2 + +2	parafoveal	0.26	0.49
2	22	♀	〃	0.08	E + E	〃	0.25	0.71
3	10	♂	〃	0.03	E + E	〃	0.31	0.85
4	19	♂	〃	0.04	−1 + −1	〃	0.21	0.50
5	17	♂	divergens	0.02	−1 + −1	〃	0.25	0.70
6	17	♂	convergens	0.1	+2 + +2	paramacular	0.19	1.53
7	15	♀	sursum-convergens	0.02	E + E	〃	0.33	1.76
8	23	♂	convergens	0.02	−4.5 + −2.0	〃	0.20	0.92
9	23	♂	〃	0.01	E + E	〃	0.13	1.51
10	23	♀	〃	0.01	−1.75 + −1.75	peripheral	0.19	2.38
11	17	♀	〃	0.04	−0.5 + −0.5	〃	0.26	1.86
12	13	♂	〃	0.02	E + E	〃	0.16	1.64
13	15	♀	〃	0.02	+2.0 + +2.0	〃	0.36	1.45

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MORPHOLOGICAL STUDIES ON THE ECCRINE SWEAT GLAND OF THE EYELID IN JAPANESE

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Introduction

Investigation on the eccrine sweat gland (E-S gland) started with Stenson (1683) who discovered the sweat pores. Since that time, numerous studies on human sweat glands have been conducted using anatomical, physiological, pharmacological, neurological and histological approaches. Many morphological studies of the human sweat gland have been reported, but these have been of a general nature and observations on the sweat glands of the body as a whole. Because of the peculiarities of the E-S glands, there has been only one report of this structure in the eyelid. This was a study of the density of the E-S glands in the facial skin by Miki[14] (1933). No description of this gland in the eyelid appears in ophthalmological or anatomical textbooks.

Therefore, a systematic study of the E-S gland of the eyelid in Japanese persons was conducted.

Materials

Ten eyelids at the margin of the orbit were separated from cadavers of Japanese individuals (Table 0). These were fixed in 10 per cent formaldehyde, embedded in celloidin, and then 20 μ horizontal and 25 μ sagittal sections were made and stained with hematoxylin and eosin.

Methods, Experimental Results and Discussion

1. Distribution of the E-S gland in the eyelid.

Density of the sweat pores. The eyelids were divided into the following 6 parts in the serial sections: inner, middle and outer parts of the lower and the upper lids. The E-S pores were counted using a microscope. The results are shown in Table 1-a. The density of the E-S glands throughout the entire body has been investigated by Krause

Table 0. Materials and Items Examined (+ examined)

Case number	1	2	3	4	5	6	7	8	9	10
Age	23	29	31	40	48	59	63	65	74	81
Sex	F	F	M	F	M	M	M	M	M	M
Plane of the serial section	·····horizontal·····				·····sagittal·····					···h
Density of the E-S pores	+	+	+	+	+	+	+	+	+	+
Depth of the glomerulus	+	+	−	−	+	+	+	−	+	+
Aglandular area	−	−	−	−	+	+	+	+	+	−
Adjacent glomerulus	+	+	+	−	−	−	−	+	+	+
Volume of the glomerulus	+	+	+	−	−	−	−	+	+	+
Dimensions of the glomerulus	+	+	+	−	+	+	+	−	+	+
Relation between the secret. and excret. ducts in glom.	−	−	−	−	+	+	+	+	−	+
Outer diameter of the duct	+	+	+	+	+	+	+	+	+	+
Length of the ducts	+	+	+	−	−	−	−	+	+	+

(1844), Rable (1902), Clarke and Lhamon (1917) and Taniguchi[18] (1956), but Miki's[14] is the only report on the density of glands in the eyelid. In his study the glands were counted in 4 parts of the eyelids: the inner and outer angles and the middle parts of the upper and the lower eyelids from 10 cadavers.

Our results agree with those of Miki that the density of E-S glands in the lower lid is about two times the number in the upper lid and the density in the middle part of the lower lid is greater, although there is considerable individual variation.

Depth of the glomerulus. The distance between the base of the glomerulus and the surface of the skin was measured vertically in each serial section and the maximum value was selected as the depth of the glomerulus.

These measurements of 1,575 glands are shown in Table 1-b.

Virchow[19] (1910), Rauber-Kopsch[16] (1934), Ito[9] (1947), Maximow[13] (1948) and Ishihara[6] (1953) reported that the glomerulus of the E-S gland throughout the body is situated in the deep layer of the cutis or the shallow layer of the subcutaneous tissue. In the eyelid, the E-S gland also seemed to be situated in these layers but the depth (0.7 mm mean value) was considerably less than elsewhere in body (1-3 mm) (Kuno[11]). The depth in the middle part of the lid was less than in the inner and outer parts of the eyelid. The shallower location of the gland in the eyelid appears to be related to the thinness of the skin of eyelid and the presence of the tarsus in the middle portion of the eyelid.

The absence of E-S glands at the lid margin. The E-S glands in the eyelid were found to be absent at the lid margin, and the border line was very sharp (Fig. 1). There is no description in the literature of this fact, although it is illustrated in Virchow's[19] textbook. The Nippon Ganka Zensho[6] which appears to have been translated from Virchow's textbock, describes E-S glands in the eyelid as decreasing in number as lid margin is approached. We have called the area without E-S glands

Table 1. Distribution of the E-S Gland in the Eyelid

1-a. Density of the Sweat Pore (per cm²)

	Upper lid				Lower lid				Mean for upper and lower lid
	Inner	Middle	Outer	Mean	Inner	Middle	Outer	Mean	
Maximum	392	328	272	392	688	848	408	848	848
Minimum	136	80	90	80	176	336	120	120	80
Mean	276	173	174	208	424	551	286	420	314

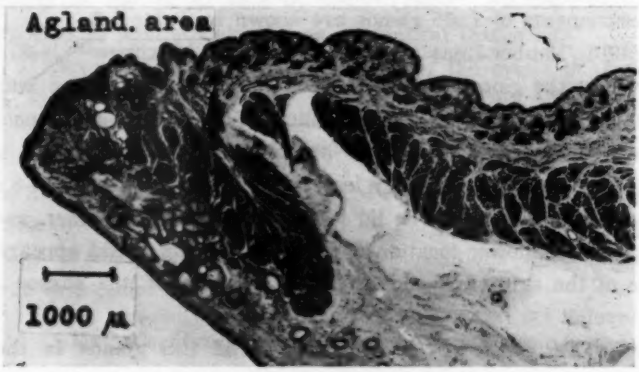
1-b. Depth of the Glomerulus (mm)

Maximum	1.75	1.25	1.75	1.75	1.59	1.25	1.25	1.59	1.75
Minimum	0.30	0.18	0.35	0.18	0.35	0.24	0.30	0.24	0.18
Mean	0.707	0.618	0.741	0.689	0.800	0.633	0.726	0.719	0.704

1-c. Sagittal Width of the Aglandular Area (mm)

Maximum	3.9	3.5	4.8	4.8	4.2	4.0	5.1	5.1	5.1
Minimum	2.0	2.8	2.2	2.0	2.1	1.8	2.2	1.8	1.8
Mean	2.91	3.21	3.62	3.25	2.94	2.92	3.50	3.14	3.17

Fig. 1. Aglandular area of the E-S gland in the eyelid.
(Lower lid of 65 yr. male, sagittal section, ×8.)



(↑: Upper limit of the E-S gland.)

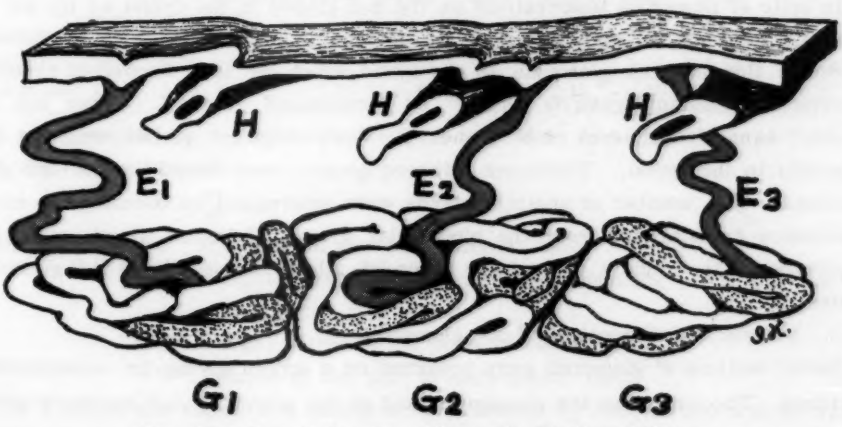
around the lid margin the **"agulandular area"** of the lid margin. Measurements of this area in 500 serial sections: are shown in Table 1-c. The mean value for the upper lids is 3.2 mm and for the lower lid is 3.1 mm. Theo uter part was about 0.5 mm wider than the middle part of the lid and the outer part of the upper and lower lids were uneven.

Fig. 2. Adjacent E-S gland in the eyelid. (Middle part in the lower lid of 81 yr. male)

Fig. 2-a. Serial section No. 432. (Horizontals. ×60.)

Fig. 2-b. Serial section No. 436. (Horizontal s. ×60.)

Fig. 2-c. Shape reconstructed.



E_{1-3}: Excretory ducts. G_{1-3}: Glomeruli. H: Hear follicle.

Table 2. Frequency of the Adjacent Glomerulus of the E-S Gland in the Eyelid

(Number of adjacent glomerulus / Number of examined glomerulus)

	Upper lid				Lower lid				
	Inner	Middle	Outer	Subtot.	Inner	Middle	Outer	Subtot.	Total
2-adjacent E-S gland	3/30	3/30	4/30	10/90	5/30	6/30	6/30	17/90	27/180
3-adjacent E-S gland	1/30	0/30	0/30	1/90	1/30	2/30	0/30	3/90	4/180
Total	4/30	3/30	4/30	11/90	6/30	8/30	6/30	20/90	31/180
Number of E-S pores	35	33	34	102	37	40	36	113	215

2. Adjacent glomeruli.

Microscopic examination of the E-S glands in serial sections revealed groups of 2 to 3 adjacent glomeruli giving the appearance or one glomerulus with 2 or 3 secretory ducts (Fig. 2). The boundaries between the glomeruli could not be distinguished. If there were a communication between the secretory and/or excretory ducts in these adjacent glomeruli, there would be one sweat gland with many excretory ducts from the one glomerulus. Clausen and Alexanderson (1929) found such E-S glands in the plantar skin, and mamed them "Zusammengesätzte Drüse mit mehreren Ausführungsgängen".

They illustrated the communications as branching and with diverticula and appendices in the secretory and excretory ducts. But the Zusammengesätzte Drüse of Clausen and Alexanderson are not the same as compound glands shown in histological textbooks.

In spite of numerous observations on the E-S glands in the eyelid by us, we have found no evidence of such communications within or between adjacent glomeruli. Therefore the adjacent glomeruli in the eyelid which we have discovered should not be termed "Zusammengesätzte Drüse" or "compound glands". They are more suitably named **„adjacent E-S glands"**. Such adjacent glands seem to occur frequently in the eyelid. Thirty-one adjacent glands were found in the 180 glands examined. The number of adjacent glands were determined by counting the number of secretory ducts arising from the glomerular group. Adjacent glands were noted frequently in the middle part of the lower lid, where the density of E-S glands was greatest.

3. Volume and dimensions of the glomeruli.

Serial sections of glomeruli were projected on a screen giving an enlargement of 100 times. The outline of the glomerulus and of the secretory and excretory portions were drawn on and then cut out of paper. The pieces of paper corresponding to each

Table 3. Volume and Dimension of the Glomerulus

3-a. Volume of the Secretory Portion (mm³)

	Distribution in group of volume (mm³)						Max.	Min.	Mean	No. of gland exam.
	↓ 0.005	0.005 0.010	0.010 0.015	0.015 0.020	0.020 0.025	0.025 ↓				
Upper lid	23	40	28	8	3	0	.02371	.00136	.0092374	102
Lower lid	17	47	33	10	4	2	.02614	.00291	.0100529	113
Total	40	87	61	18	7	2	.02641	.00136	.0096650	215

3-b. Volume of the Proximal Portion of the Excretory Duct (mm³)

	Distribution in group of volume (mm³)						Max.	Min.	Mean	No. of gland exam.
	↓ .0010	.0010 .0025	.0025 .0050	.0050 .0100	.0100 .0150	.0150 ↓				
Upper lid	9	41	29	22	1	0	.01243	.00025	.0033556	102
Lower lid	15	42	45	9	1	1	.01521	.00025	.0027551	113
Total	24	83	74	31	2	1	.01521	.00025	.0030400	215

3-c. Volume of the Glomerulus (mm³)

	Distribution in group of volume (mm³)						Max.	Min.	Mean	No. of gland exam.
	↓ 0.005	0.005 0.010	0.010 0.020	0.020 0.030	0.030 0.040	0.040 ↓				
Upper lid	8	24	46	16	8	0	.03561	.00291	.0151172	102
Lower lid	8	22	57	21	4	1	.04385	.00456	.0155556	113
Total	16	46	103	37	12	1	.04385	.00291	.0153475	215

3-d. Dimensions of the Glomerulus (mm)

	Upper lid				Lower lid				Mean for upper and lower lid
	Inner	Middle	Outer	Mean	Inner	Middle	Outer	Mean	
Transversal D.	0.45	0.63	0.50	0.53	0.59	0.67	0.56	0.61	0.58
Vertical D.	0.50	0.30	0.41	0.40	0.52	0.36	0.44	0.44	0.42
Antero-post. D.	0.31	0.22	0.29	0.27	0.28	0.23	0.31	0.27	0.27

gland, were weighed on a chemical balance. The volume of a respective part of the gland is given by formula,

$$V = \frac{w}{W} \times D$$

where D : thickness of the serial section (mm).

w : Weight of the piece of paper corresponding to the part of the gland (gm).

W : Weight of 10×10 cm piece of paper (gm).

V : Volume of the part of each gland (mm).

One hundred and eighty E-S glands were measured by this method and the values are shown in Table 2. Thirty one adjacent glands were included among a total of 180

glands. Adjacent glands were considered to have glomeruli of equal volume.

Volume of the secretory duct. The volume of this part of the glomerulus was measured by the above method. The results are shown in Table 3-a.

Volume of the proximal portion of the excretory duct. The volume of this part of the glomerulus was measured by the above method. The results are shown in Table 3-b.

Volume of the glomeruli. The volume of the whole glomerulus was measured by the above method. The results are shown in Table 3-c.

Dimensions of the glomerulus. The eyelid was divided into 6 parts in the serial sections as described in paragraph 1-a. In each part of upper lid, there were measured 25 glands, and in the lower lid 50 glands, from 8 cadavers. Transversal, vertical and anterior-posterior dimensions were measured in a total of 1800 glomeruli (Table 3-d).

The dimension of the E-S gland is reported to range from 0.3-0.4 mm: the largest was 2-7×1-3, the smallest 0.06 mm, according to Rauber-Kopsch[16] (1934). Maximow[13] (1948) gave similar measurements. According to Kuno[11] (1946) the measurements are 1/3-1/4 mm in diameter and 1/50-1/60 mm^3 in volume. Taniguchi[18] (1956), who has investigated the accessory organs of the skin, gave the mean volume of the frontal E-S glands as 0.014504 mm^3 in a 15 year old female, 0.020740 mm^3 in 18 year old female, 0.017160 mm^3 in an other 18 year old female, 0.023558 mm^3 in a 19 year old female and 0.017160 mm^3 in a 31 year old male. As mentioned above, there have been several reports on the volume of the E-S gland in the body as a whole, but no data on the E-S gland of the eyelid. It seems that there is no difference between the values for glands throughout the body and those for the glands of the eyelid, in spite of characteristically distinct morphological shapes. As shown in Table 3-d, the transversal dimension was largest and the anterior-posterior dimension was shortest.

According to the above mentioned facts and observations with wax reconstruction models[5], the shape of the glomeruli of the E-S gland in the eyelid was found to be flat in the anterior-posterior direction and long and elliptical in the transversal direction. Especially in the middle part of the lids, the transversal dimension was greatest, so the glands may be appropriately called spindle or club-shaped. The typical shape of the E-S gland in the eyelid is illustrated in Fig. 3.

The cutis and subcutaneous tissues are thin and seen to have a regular lammellated disposition in the horizontal plane because of the existence of the tarsus.

There have been no previous descriptions of the characteristic shape of the E-S gland in the eyelid.

Relationship of the structural parts of the glomeruli. The glomerulus may be divided into 3 parts: (1) secretory (2) excretory and (3) other elements such as nerves, blood vessels and connective tissue. The relative volumes of these parts in the glomerulus are 63 per cent, 20 per cent and 17 per cent or a ratio of about 3:1:1 respectively.

On the other hand, according Ito and Ota[10] (1950), glomeruli may be classified into 3 forms according to the relationship of secretory and excretory ducts as follows:

Fig. 3. A typical E-S gland in the eyelid.
(Middle part in the lower lid of 29yr., male.)

Fig. 3-a. Microphotograph of the E-S gland. (horizontal section, ×60.)

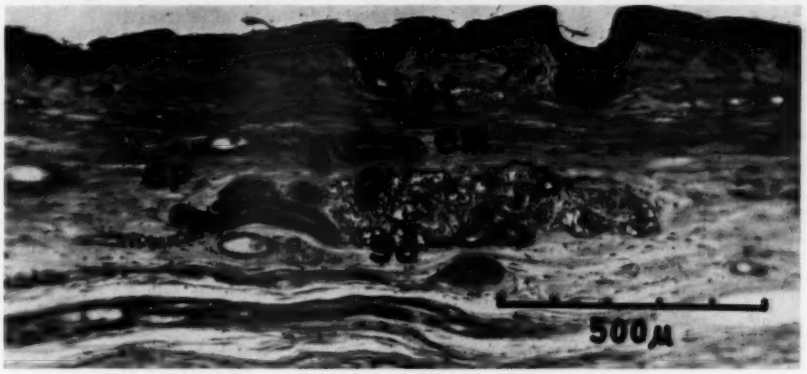
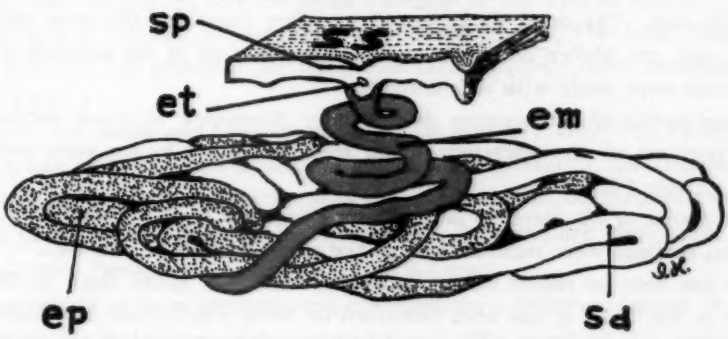


Fig. 3-b. Wax reconstruction model of the E-S gland.

Fig. 3-c. Shape reconstructed.



em: Middle portion of the excretory duct. **sp**: sweat pore
ep: Proximal portion of the excret. duct. **ss**: skin surface
et: Terminal portion of the excret. duct.

Table 4. Outer Diameter of the Ducts

4-a. Secretory Duct (μ)

Upper lid				Lower lid				Max.	Min.	Mean
Inner	Middle	Outer	Mean	Inner	Middle	Outer	Mean			
41	40	41	41	44	42	42	43	81	19	42

4-b. Proximal Portion of the Excretory Duct

31	30	31	31	31	30	32	30	72	12	31

4-c. Middle Portion of the Excretory Duct

34	33	35	34	33	33	34	33	53	14	34

Form 1: Excretory ducts are not part of the glomerulus, and the glomerulus consists only of a secretory duct. This form may be seen in the apocrine sweat gland.

Form 2: The glomerulus is made up of the both excretory and secretory ducts which do not intertwine.

Form 3: The glomerulus is made up of both excretory and secretory ducts which intertwine.

A total of 1,125 glands were observed: 25 glands in each part of the upper lid and 50 glands in the lower lid, from 5 cadavers. No Form 1 glomeruli were found. There were 9.3 per cent of Form 2 and 90.7 per cent of Form 3 glands found. It was expected that Form 1 glomeruli might be found in the lid margins which have Moll's glands, but none was observed.

4. Outer diameter of the E-S gland duct.

Secretory duct. The outer diameters of secretory ducts in 3,000 cross sections were measured and are shown in Table 4-a. Measurements of the smallest diameter in cross sections were made with a micrometer.

Proximal portion of the excretory duct. Outer diameters in cross sections of the proximal portions of the excretory ducts were measured by the same method as in the above (Table 4-b).

Middle portion of the excretory duct. Outer diameters of this portion in cross and longitudinal sections were measured using the same method (Table 4-c).

There has been no report on the diameter of the E-S gland duct in the eyeilds. Elsewhere in the body, it has been described as being 0.05-0.3 mm by Ishizawa[7] (1956). An inner diameter of 19 μ for the secretory duct and 21-25 μ for the secretory duct of the E-S gland in the dorsum of the terminal phalanx have been reported by Ito[8] (1949), who also pointed out the isthmus and ampulla in the duct.

Table 5. Length of the Ducts of the E-S Gland

5-a. Secretory Duct (mm)

	Distrib. in group of length(mm)				Maximum	Minimum	Mean	No. of gland examined
	—10	10-20	20-30	30—				
Upper lid	83	19	0	0	19.6	1.3	7.2	102
Lower lid	87	25	1	0	20.5	1.7	7.4	113
Total	170	44	1	0	20.5	1.3	7.3	215

5-b. Proximal Portion of the Excretory Duct

Upper lid	100	1	0	0	13.6	0.8	4.3	102
Lower lid	107	7	0	0	14.3	0.4	4.0	113
Total	207	8	0	0	14.3	0.3	4.2	215

5-c. Ducts in Glomerulus

Upper lid	42	51	9	0	27.4	3.3	11.5	102
Lower lid	56	41	15	1	32.0	2.6	12.7	113
Total	98	92	24	1	32.0	2.6	12.1	215

The secretory ducts have the largest diameter, the proximal portion of the excretory duct the smallest. There are locational and individual variations in thickness. Elsewhere these alterations in the calibre of the duct may be called "isthmus" or "ampulla", but in the eyelid they are merely variations in the diameter of the duct.

5. Length of the duct of the E-S gland.

The secretory duct (*Table 5-a*). The length of the secretory duct was calculated from the volume of each glomerulus as shown in paragraph 3 and mean diameters of the duct in each part of the cadaver were measured as in paragraph 4.

The proximal portion of the excretory duct (*Table 5-b*). The length of the duct was calculated from the volume and the mean diameter of the duct as above.

Total length of the duct of the glomerulus (*Table 5-c*). The total length of the secretory duct and the proximal portion of the excretory duct were calculated and are shown in Table 5-c.

Length of the middle and terminal portion of the excretory duct. According to experiments with wax reconstruction models, the depth of the glomeruli, the anterior-posterior dimension of the glomeruli, the thickness of the epidermis, and the tortuosity of the ducts, the length of the middle and terminal portions of the excretory duct were estimated to be 0.5-1.0 mm (maximum 2 mm, minimum 0.3 mm).

The tortuosity of these portions was considerable. Previous reports of the length of the duct in the body as a whole are as follow: The length of the secretory ducts was about 6.5 mm. The length of the excretory ducts was not constant on account of the thickness of the skin (Ishizawa[7]). The length of the excretory

duct was 1-3 mm from the glomerulus to the skin surface (Kuno[11]). The total length of the sweat gland duct was 0.7-1.7 mm in the dorsum of the terminal phalanx (Ito[8]). Two thirds of the glomeruli consisted of secretory duct and the remainder was excretory duct (Maculeod and Muende[12]). These reports have been so fragmentary that comparison with the results of this study are not entirely adequate.

Conclusions

Morphological studies on the eccrine sweat (E-S) gland in the eyelid of Japanese using histological methods have been conducted. The results are as follow:

1. The average density of E-S pores in the eyelid was 314 per cm^2 but the density in the lower lid was about twice that in the upper lid. The average depth of the E-S gland in the eyelid from the skin surface was 0.7 mm, which was considerably less than elsewhere in the body.

2. The E-S gland was found to be absent at the lid margin. This "*aglandular area*" averaged 3.2 mm in width. In the eyelid, groups of 2 or 3 adjacent glomeruli were found in 31 of 180 glands examined. They gave the appearance of one glomerlus with 2 or 3 secretory ducts. It is proposed that they be termed "*Adjacent Glomeruli*"

3. The average volume of the glomerulus was found to be 0.0153475 mm^3 in the 180 glomeruli examined. The relative volumes of the three parts in the glomeruli: secretory and excretory ducts and other elements, are about 3:1:1. The average dimensions of the E-S glomerulus as measured in a total of 1,800 glands were $0.58\times 0.27\times 0.42$ mm. The usual shape of the glomerulus was found to be flat in anterior-posterior direction and long and elliptical in the transversal direction.

4. The average outer diameters of the E-S gland duct in 3,000 cross sections were 42μ for the secretory portion, 31μ for the proximal portion, and 34μ for the middle portion. Classification of the glomerulus according to the relationship between the secretory and excretory ducts reveals that no Form 1 glommeruli were found, 9.3 per cent were of Form 2 and 90.7 per cent were of Form 3. The average length of the secretory duct was 7.3 mm, the proximal portion of the excretory duct 4.2 mm, the terminal portion of the excretory duct 0.5-1.0 mm and the average total length of the duct was about 12 mm.

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A STRAIN OF FIBROBLASTIC CELLS FROM THE RABBIT CORNEA AND ITS SUSCEPTIBILITY TO HERPES SIMPLEX VIRUS

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In the previous paper, morphology of the epithelial and endothelial cells from the rabbit cornea in tissue culture was reported. In the following report, a method of establishing a strain of fibroblastic cells from the rabbit cornea, and some factors conditioning growth of the cells were described. Herpes simplex virus produced the cytopathogenic effect on this strain.

Meterials and Methods

Rabbit corneas were obtained from an adult albino rabbit immediately after death. Thin slices of superficial layer were shaved and chopped into fragments approximately 1 mm square and placed on a coverslip coated with plasma clot. After inserting them in roller tubes, 1.2 ml of nutrient medium was added. The medium consisted of 80% of Hanks BSS containing 0.1% yeast extract and 0.5% lactalbumin hydrolysate (subsequently referred to as YLH) and 20% of horse serum. Penicillin and streptomycin were added to the medium at a concentration of 500 u/ml and 100 mcg/ml respectively. Cultures were incubated at 37.5°C and rotated (12rph) in a slanted position.

In order to subcultivate the grown cells, vercenate or trypsin (1:300 NBC) was used. Both were dissolved in saline buffered with phosphate.

Short culture tubes with flattened surfaces, TD 15 flasks (Designed by H. Katsuta:

Institute for Infectious Diseases) and square bottles (200 ml) were used for subcultivation.

HF-HL3 strain of herpes virus was supplied by the Institute for Infectious Diseases, University of Tokyo.

For histologic examination, the cells on a coverslip were fixed in methanol or Bouin's fixative and stained by Giemsa or Harris hematoxylin and eosin.

In order to measure the growth rate of the cells, propagated cells were treated with vercenate and after centrifugation the supernatant was replaced with 0.05% citric acid solution containing crystal violet. The number of the cell nuclei was enumerated in a hemocytometer.

Results

Subcultivation. In the primary cultures, proliferated cells consisted mainly of epithelial cells which showed a polygonal shape and pavement like appearance. For the purpose of obtaining the pure culture of epithelial cells the original explants were removed on the 5th day, when the whole amount of fluid medium was renewed.

On the 7th day, 7 tubes which showed good growth were selected and fluid medium was replaced with 1.2 ml of 0.02% vercenate solution. Incubated at 37.5°C, the cells in plasma clot were dispersed by gentle agitation. The fluid phase was removed after the centrifugation at 1000 rpm for 5 minutes, and the cells were resuspended in 4 ml of medium from the previous culture. Each 1 ml of the cell suspension was transferred to a short culture tube with flattened surfaces.

Twenty four hours after the incubation, most of the floating cells attached to the glass surface. Between the round cells, there could be seen some cells which flattened out.

After 48 hours, proliferating cells on the glass increased in number and in the portion where good growth occured, a monolayer of cells was observed. When they attached to the glass in a clump, they grew out radially and showed a colony like appearance in bacterial cultivation.

In this time, and on the 6th day as pH of medium became acid, two thirds of the whole volume was replaced with fresh medium.

On the 8th day the culture was treated with a solution of vercenate. After the centrifugation the cells were resuspended in 1.5 ml of new medium and transferred to a TD 15 type flask, then 0.5 ml of old medium was added.

In the 2nd subculture, growth was observed like in the previous culture. The cells grown in compact arrangement showed an epithelial like appearance and between them those with elongated cytoplasm and plasmic extensions were found.

As the 3rd subculture a culture in TD 15 was distributed into three flasks of the same type. Since the 2nd subculture, penicillin and streptomycin in the medium were reduced to 250 u/ml and 50 mcg/ml respectively.

At the 4th subculture, cells in each of TD 15 were transferred to a square bottle with 6 ml of medium. Fluid medium from the previous culture was added to new cultures in one third to one fourth of the total volume of the medium, and when pH decreased, two thirds to three fourths of the medium was renewed. The cultures of the 4th and 5th subcultures exhibited poor proliferations, so they were maintained for a relatively long time, and the cells in one flask were not divided into several but transferred to one. The rate of growth recovered in the 6th subculture.

In the 7th subculture, the cells were grown on a coverslip inserted in a flask and stained by Jacobson's stain for microscopic examination. All cells in the preparation exhibited so called fibroblastic morphology and none of the epithelial cells could be found.

Since the 10th subculture, vercenate was replaced with 0.1 to 0.05% of trypsin. The cells are now maintained in continuous cultures (80th subcultivation in August, 1960) by the use of 20% of horse serum in YLH and trypsinized once a week. When a culture is distributed into four new vessels growth is sufficient for subcultivation usually within 5 to 6 days.

Morphology of the cells.

In the early days of cultivation there existed sparcely distributed cells of triangular or spindle shape with needle like processes, and none of them were polygonal like the epithelial cells.

When they grew out of the clump, in the central portion, they showed a pack arrangement but peripherally they were spindle shaped. (Fig 2)

In the late stage, the cells increased in number. The cytoplasm was elongated and the spindle shape became remarkable. They conjugated with each other end to end, aligned in parallel and formed swirl like configurations. (Fig. 3)

The nucleus was elipsoid and contained one to three nucleoli. These findings were characteristic for the fibroblastic cells.

Sometimes cells with a large nucleus were seen. (Fig. 4) and mitosis could be found more frequently in young cultures.

By means of the phase contrast microscopy, the cell surface became still clearer and mitochondria and fat granules were easily visible. (Fig. 5)

When the cells were cultivated on coverslips coated with plasma clot they became more elongated and similar to the fibroblast found in the primary culture of corneal stroma. (Fig. 6)

Some factors conditioning the growth.

1. Horse serum in medium: A cell suspension was obtained from the 7th subculture and an equal volume of it was transferred to the short test tubes. Horse serum added to make the final concentration of 10, 20, 30 and 40% in medium and pH was adjusted to 7.4. Each group was composed of three tubes.

Seven days after, cultivation was stopped, the cell nuclei were enumerated. The

Figs. 1 to 6. Fibroblastic cells of the 7th subculture.
Figs. 1 to 4 and 6 are photographed with the same magnification. Jacobson's stain.

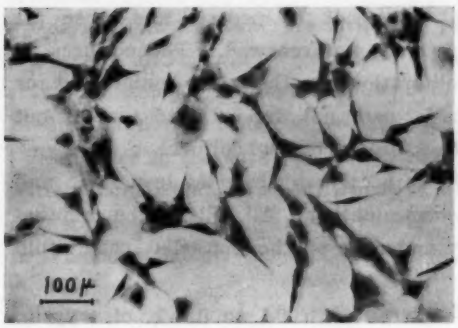


Fig. 1. After 1 dav of cultivation.

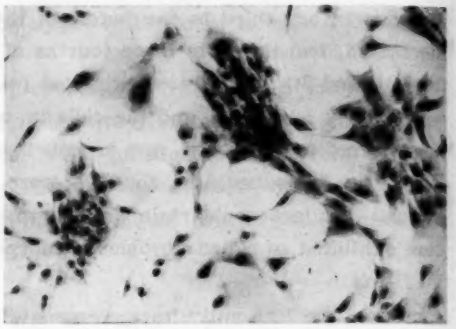


Fig. 2. After 2 days of cultivation. Growth of the cells from the clumps.

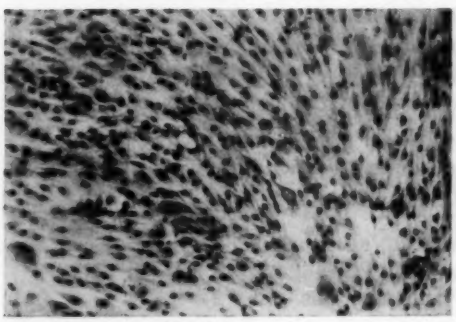


Fig. 3. After 4 days of cultivation. The cells are aligned in parallel.

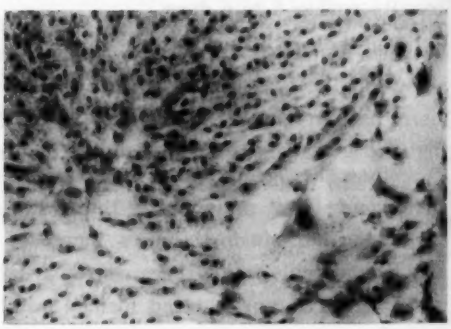


Fig. 4. After 12 days of cultivation. A giant cell is seen.

Fig. 5. After 3 days of cultivation. Dark phase contrast.

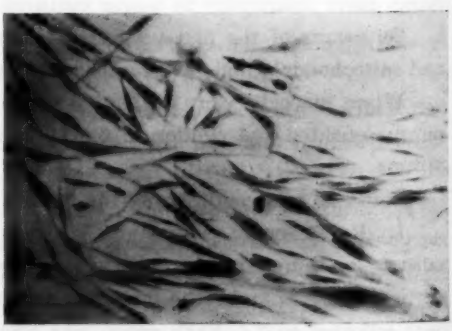


Fig. 6. After 3 days of cultivation. The cells grown on plasma clot.

Figs. 7,9 and 10 are photographed with the same magnification. Hematoxylin and eosin.

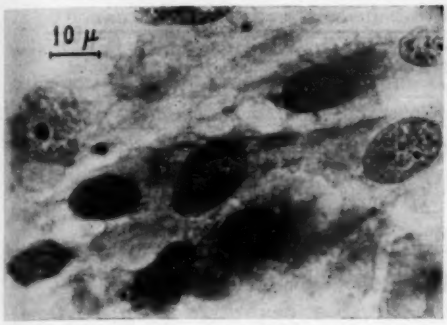


Fig. 7. Normal fibroblastic cells.

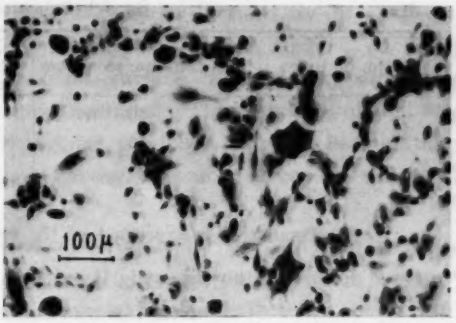


Fig. 8. The cells infected with herpes simplex virus, 24 hours.

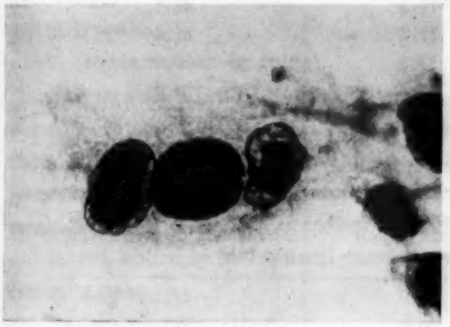


Fig. 9. After 24 hours of infection. Type A intranuclear inclusion.

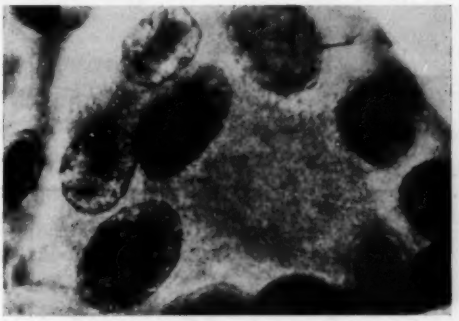
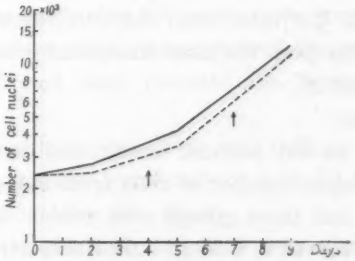


Fig. 10. After 24 hours of infection. A multinucleated cell.

Fig. 11. Growth curve of the cells.



——30% horse serum in YLH
·····20% horse serum in YLH
Arrows mean the renewal of the medium.
Points represent the average number of cell nuclei in 3 tubes.

Table 1. Effect of the Concentration of Horse Serum on the Cells from the 7th Subculture

No. of tubes	Per cent horse serum	Initial population. Thousands of cell nuclei per tube	Population after 7 days. Thousands of cell nuclei per tube
3	10	104	150
3	20	104	252
3	30	104	338
3	40	104	311

Population is expressed by the mean value.

Table 2. Effect of the Concentration of Horse Serum on the Cells from the 22nd Subculture

No. of tubes	Per cent horse serum	Initial population. Thousands of cell nuclei per tube	Population after 7 days. Thousands of cell nuclei per tube
3	10	17.3	46.2
3	20	17.3	81.8
3	30	17.3	139.0
3	40	17.3	64.8

Population is expressed by the mean value.

Table 3. Effect of Old Medium

No. of tubes	Compornents of medium (ml)		Initial population. Thousands of cell nuclei per tube	Population after 7 days. Thousands of cell nuclei per tube
	fresh	old		
3	1.5	0	54.0	271
3	1.0	0.5	54.0	258
3	0.5	1.0	54.0	99

Population is expressed by the mean value.

result is shown in Table 1.

It seemed to be, that 30% horse serum in YLH was the most beneficial to cell growth. Growth curve of the cells from the 8th subculture was shown in Fig. 11. The best growth of the cells from the 22nd subculture was obtained in the group of 30% horse serum too (Table 2).

2. Conditioned medium:

The effect of adding an old medium conditioned by a mother culture to a new culture was tested. The equal number of cells from the 11th generation were distributed into short test tubes and three groups were made. In each of the groups the amount of the old medium was 0, 0.5 and 1.0 ml respectively in the whole volume of 1.5 ml. On the 4th day of culture medium was renewed by a fresh one. The number of cell nuclei in each group on the 7th day was shown in Table 3. Cellular growth was inhibited in the 3rd group. Not any growth promoting effect was found in the conditioned medium.

3. Trypsinization:

Though vercenate was used in the earlier generations, the availability of trypsin was tested. Four groups of it, 1%, 0.5%, 0.25% and 0.1% were made. To five cultures, which formed a cellular sheet on the glass surface, those of trypsin and vercenate were added after withdrawal of the fluid medium. They were incubated at 37.5°C and it took 4 minutes for 1% trypsin, 7 minutes for 0.5% trypsin, 10 minutes for 0.5% trypsin, 12 minutes for 0.1% trypsin and 20 minutes for vercenate solution to make cells free from the glass.

More homogenously dispersed cell suspensions were obtained by trypsin than by vercenate, and by the latter the cells were apt to clump. Higher concentrations of trypsin employed in the experiment seemed not to have any deleterious effect on the cells. But now 0.05% trypsin is used, since this concentration proved to be sufficient for the purpose. Chymotrypsin (Mochida Co. Ltd. in Japan) which has been used in eye surgery was available too and 0.02% of it seemed to be nearly equivalent to 0.05% trypsin.

4. Preservation in cold:

Cultures in TD 15 type flasks which had been cultured for 5 days were stored in a refrigerator at 2° to 3°C. Within 24 hours round cells appeared in the cellular sheet and they increased in number as time of storage passed. Two weeks after a monolayer of the cells changed into a network of cells but when the cultures were reincubated, new growth occurred from the cells which had remained on the glass.

In the cultures maintained for four weeks, though poorly, recovery of the cells could be seen.

Herpes simplex virus:

Cultures were prepared from the 18th subculture in the test tubes in which coverslips were inserted. After 3 to 5 days the medium was replaced with 1.2 ml of YLH containing 10% chicken serum, and this was followed by the addition of 0.05 ml to 0.2 ml of appropriately diluted stock virus. They were incubated at 36°C.

In the group of cultures containing 0.2 ml of virus, round refractile cells appeared after 24 hours. After 48 hours other groups showed the same change. The cells on the coverslips were stained and they revealed the degeneration described in the following.

After 72 hours from the culture inoculated with 0.05 ml of virus, 0.6 ml of fluid phase was transferred to a square bottle culture with 5.4 ml of 10% horse serum in YLH and cytopathogenic effects were also found.

By the same procedure the effect of the virus was observed in the 3rd culture and the cells stained by hematoxylin and eosin were shown in Figs. 8 to 10.

Cytoplasm of the changed cell lost its processes and became round. The multinucleated cells were frequently encountered.

In the nucleus, chromatin was condensed in the nuclear membrane and a rarefied

zone, which surrounded homogenous pinkish blue masses, appeared underneath the nuclear membrane. Later, these masses shrank, became more acidophilic and type A inclusions were formed.

The nuclei contained in multinucleated cells exhibited various kinds of stages of lesion.

Discussion

Several kinds of epithelial cells have been established as strains, but about the cornea none has been reported except Fowle's work which described, that the epithelial cells had been maintained through three subsequent passages.

Attempting to obtain a strain of epithelial cells from the cornea, the author cultivated thin slices of superficial layer of the rabbit cornea and subcultivated the grown cells. Although the original explants had been removed on the 5th day of the primary culture in order to eliminate the growth of fibroblasts, epithelial cells disappeared during the courses of subcultivation and finally a strain of fibroblast was established. It was thought, that though in the primary cultures the propagated cells seemed to be composed of mainly epithelial cells, fibroblasts had mingled and overgrew the former.

It might be concluded, that fibroblasts were derived from the stromal cells in the thin layer of stroma in the explants.

The usual methods used in cultivations of the strain such as HeLa, Chang's or FL cells etc. could be applied for this strain.

The cells had the maximal growth rate in YLH with 30% horse serum at the 8th (2 months) and also at the 22nd generation (5.5 months), although they had been maintained in continuous cultures for three and a half months in YLH with 20% horse serum. Adaptation of the maximal growth rate of the cells to the medium with 20% horse serum used constantly for successive cultures, could not be found during the period. In procedure for subcultivation, addition of old medium to the new culture was stressed in Earle's work of isolating a single cell. From this result however, it was not essential, when the strain was treated as a massive culture.

In preparing the cell suspension for subcultivation, 0.05% trypsin (NBC 1 : 300) was rather superior to 0.02% of vercenate in making more homogenously dispersed suspension.

In attempt to store strains, preservation at subzero-temperature by medium containing glycerol has been succeeded by several investigators. It was possible to preserve this strain in a refrigerater (2° to 3°C) as long as four weeks though recovered cells were markedly decreased in number.

Infected with herpes simplex virus the cells showed the alterations characteristic for this virus. The effects of virus on the cells were transferred through three serial passages and dilutions of virus suspension were 0.05/1.25, 0.6/6 and 0.15/1.5 respectively.

The possibility that herpes virus propagated in this strain of the cells was confirmed. It is interesting, that fibroblasts derived from the cornea are susceptible to herpes simplex virus. It does not, however, imply that this virus has pathogenic effects on the stromal cells in herpetic keratitis, because the behavior of cells in vitro to viruses is different from that in vivo.

Summary

The thin slices of superficial layer of the corneas from an albino adult rabbit were cultured in vitro. The fibroblasts overgrew the epithelial cells during the subcultivation and were established as a strain.

The cells of this strain showed the maximal growth rate in 30% horse serum in Hanks BSS, containing 0.5% lactalbumin hydrolysate and 0.1% yeast extract.

A growth promoting effect was not obtained by the use of an old medium or cell conditioned medium.

For dispersing the cells in the procedure of subcultivation 0.05% trypsin was superior to 0.02% vercenate solution.

The cells could survive for 4 weeks at 2° to 3°C.

Infected with herpes simplex virus they exhibited the characteristic alterations.

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EXPERIMENTAL STUDIES ON RELATION BETWEEN CONJUNCTIVAL LYMPHVESSELS AND INTRAOCULAR LYMPH STREAMS IN THE RABBIT'S EYE

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Introduction

While the detailed studies on the conjunctival lymphvessels have been made by Arnold and others, the problem of intraocular lymph streams, especially anterior

aqueous passage, and their relationship to aqueous veins remains unknown. This problem is dealt with in this paper.

Experimental methods

In the following investigations a number of albino rabbits of 2 kg body weight are used.

1. Observation of conjunctival lymphvessels.

At first the physiological injection method of using eye drops of 1% brilliant cresyl blue as a physiologic solution was used and the vital eye of the rabbits were inspected with the Baush and Lomb type of binocular corneal microscope. In some cases the stretched specimens of the stained conjunctiva were made to be inspected by ordinary microscope.

It should be noted, however, that by this method of eye drop instillation the deep lymphvessels could not be observed enough, then a system of "stick-injection" of Indian ink was devised for the purpose of observing the deep lymphvessels and the histological specimens. It was recognized, that Indian ink injected in a specimens of the conjunctiva is very helpful to this experiment, because the other stains were apt to be oedematous stagnated and penetrated into tissue spaces resulting in disturbed transparency of the tissues which inhibited precise inspections.

After extirpating the eyeball, Indian ink was injected into the bulbar conjunctiva near the corneal limbus using a common syringe with 1/5 needle. It was noted, by the use of binocular corneal microscope, that the presence of Indian ink was confirmed in the lymph streams. Therefore, a series of sections were made from such a specimen by a method of alcohol fixation and paraffin embedding. Furthermore, the method of Berlin blue reaction and nuclear stain using alum carmine to these sections was applied in order to inspect histologically.

2. Observation of aqueous passages.

General anesthesia was applied to the albino rabbits with 15 ml of 20% urethane intramusculary. The rabbits were then fixed without pressing their necks, and the vessels in their corneal limbus were observed. 1% trypan blue solution was then injected into the anterior chamber in order to observe the anterior aqueous passages.

For the purpose of histological examination Kishimoto's procedure was, of necessity, followed. A syringe needle was placed 2 mm distant from the corneal limbus and was inserted deeply into the anterior chamber at the site facing the pupil through the corneal parenchyma. After removing the aqueous humor, a solution of ferric citrate was injected into the anterior chamber under 50 mmHg pressure for a period of 30 to 60 minutes. Immediately after the needle was removed, the injection point must be cauterized in order to prevent further outflow of aqueous humor. The used ferric citrate solution is as follows:

Ferric citrate	1.0 g.
Sodium chloratum	0.8
Sodium bicarbonicum	0.3
Aqua destillata	100.0

pH : 7.6, Lowering degree of freezing point : 0.64.

The eyeballs were immediately extirpated, and then these eyeballs were soaked in a solution consisting of the same quantities of 2% pottasium ferrocyanatum and 1% hydrochloric acid for about an hour to complete the Berlin blue reaction process. After the reaction process, the eyeballs were washed with Ringer's solution, and a number of serial paraffin sections 10 μ thick were prepared from these eyeballs by the method mentioned just above. This new method was better than the ordinary one, by which the Berlin blue reaction process was done on the prepared sections, for the ferric reaction proceedes more strongly without ferric penetration into tissues.

Result

1. Conjunctival lymphvessels.

When 1% brilliant cresyl blue solution is applied to the conjunctival sac, it works in the epithel cells of the conjunctiva and cornea separately or as a group being stained pale blue with or without a tint of violet. In case where the process of staining is good, the conjunctival and episcleral connective tissues are well stained and connective tissues of the lymphvessel-walls along the small blood vessels appear pale blue. In the case of extreme staining, blood vessels are covered completely with stain and are reduced to blue lines. These phenomena may be considered as perivascular lymph spaces (Fig. 1).

Furthermore, the relative deep lymphvessels are stained blue and are several times as big as blood vessels in width and make an irregular meshwork with no sharp boundary. Some of them run independently from blood vessels.

Fig. 1.

Stretched specimen of bulbar conjunctiva. Perivascular lymph space. Vital staining of brilliant cresyl blue.

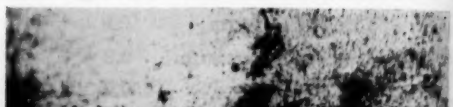
In the specimen prepared with Indian ink, macroscopic observation detected small black lines expending in all directions, starting from the stagnation of the Indian ink at the site of injection. In case where a microscope of high power was used to observe the stretched specimens of the conjunctiva, Indian ink particles can be recognized on the vessels and contracted part of the vessel which seems to correspond to the valve (Fig. 2, A and B).

It is noted, that superficially and

Fig. 2, A.

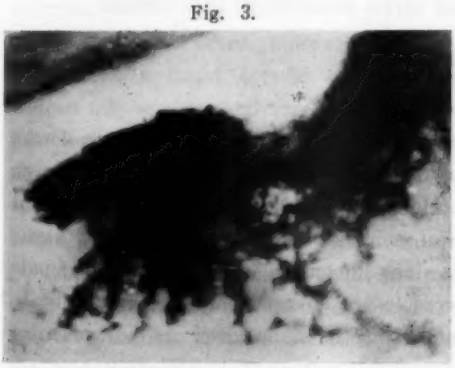
Specimen of Indian ink stick-injection. Macroscopic finding. C: Cornea. I: Indian ink.

Fig. 2, B.



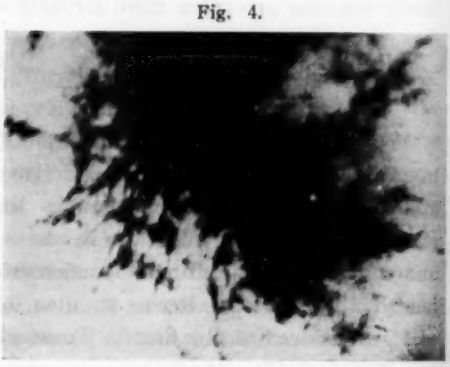
The same. Stretched specimen of bulbar conjunctiva. Microscopic finding. →: Contriction corresponding to valve.

Fig. 3.



The same Superficial lymphatic network.

Fig. 4.



The same. Deeply situated lymphatic network.

deeply situated lymphvessels appear separately or together depending on the depth of the inserted needle (Fig. 3 and 4).

When the eye drops of the stain solution are applied before the injection of Indian ink, the deposits of the latter can be easily found in the stained vessels.

When Indian ink is still more deeply inserted, black lines can be noted expending in all directions from the injected site. According to the histological observation, it can be noticed, that a portion of the injected Indian ink appears in the tissue spaces and the rest are situated in the subconjunctival or episcleral layer. When the Indian ink is injected into the lymphvessel, it is in part subjected to the phagocytosis of its endothelium and the remainder is deposited on the vessel wall. The wall is of one layer of the endothelium with no muscular cells. Though, in some cases, lymphvessels are as big as the neighbouring veins, the former is usually but a fraction of the latter.

2. Aqueous passages.

The vessels along the corneal limbus contain the transparent fluid in the upper anterior region from the cornea. When the trypan blue solution is injected into the anterior chamber, it can be noted, in the upper anterior region of the bulbar conjunctiva,

relatively big vessels are stained in pale blue, as the stain transfers upperwards. These vessels run from the anterior side to the upper side along the corneal limbus and gradually depart from it almost with no winding. The origin of the vessel seems to be at the sclera. It is noted, however, that the exact origin can not be identified from this result. There is no abnormal condition to be found in the small vessels surrounding the corneal limbus under this condition. These blood vessels are considered to be from the anterior chamber angle or ciliary body, and in addition, there are superficial blood vessels which run from the conjunctiva to the cornea (Fig. 5, A and B).

When the eyeball is compressed from the temporal side, the above mentioned blue tone becomes strong. At the same time there appear small deep vessels, stained in blue, running at 3 mm from at right angle to the corneal limbus, and joining one of the big vessels just mentioned above. If the eyeball is more strongly compressed, all the blood vessels on the eyeball including the pericorneal ones, change from red to a blue tone because of the stain solution, except for the superficial vessels running to

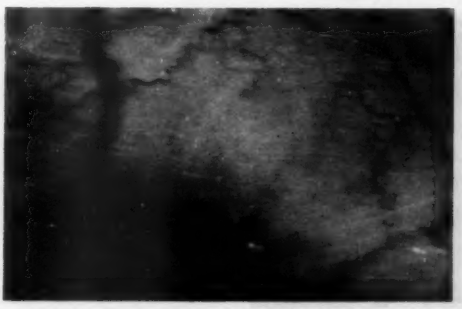
Fig. 5, A.

Vital observation of the aqueous vein. Normal blood stream of the corneal limbus.

Fig. 5, B.

The same. Model. a.: Aqueous vein. b.: Deeply situated aqueous vein. c.: Superficial conjunctival blood vessel.

Fig. 5, C.

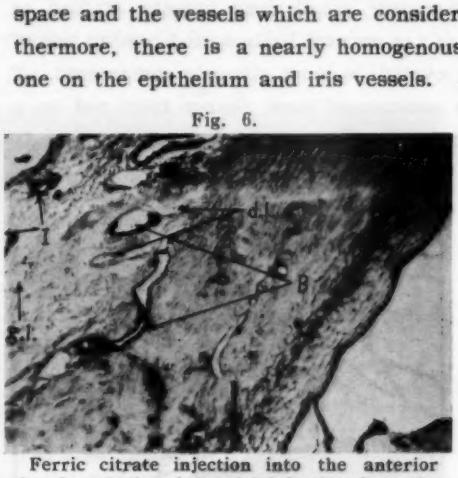


The same. Due to compression (a) and (b) disappeared filled with transparent aqueous humor, and (c) became marked.

the cornea. It is noted, however, that the stain solution is again replaced with blood, when compressing the eyeball is discontinued. In the case where the eyeball is compressed without the previous injection of trypan blue into the anterior chamber, the pericorneal vessels are filled with tranparent aqueous humor which results in the disappearence of the normal blood vessel feature (Fig. 5, C).

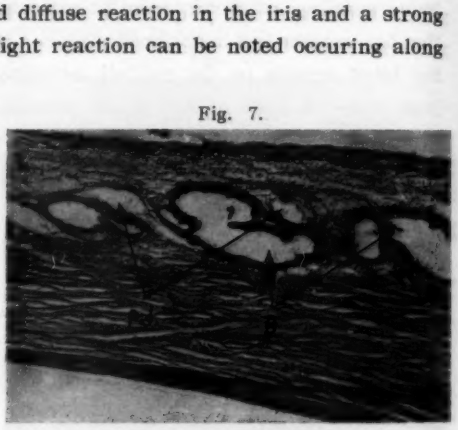
It can be ascertained through histological observation, that there exists rarely a very slight ferric reaction in the epithelium of the conjunctiva and cornea, and that, in some cases, there is a slight reaction in two-thirds of the corneal parenchyma from the surface and a strong one in the deeper layer such as the Descemet's membrane and the endothelium respectively. The reaction can be also observed in Fontana's space and the vessels which are considered to correspond to Schlemm's canal. Furthermore, there is a nearly homogenous and diffuse reaction in the iris and a strong one on the epithelium and iris vessels. A slight reaction can be noted occuring along

Fig. 6.



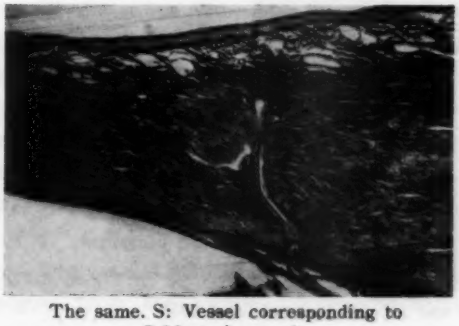
Ferric citrate injection into the anterior chamber, Indian ink stick-injection into the conjunctiva. Ferric reaction. Alcohol fixation. Paraffin section. Alum carmine nuclear stain. g.l.: Great lymphvessel. d.l.: Deeply situated lymphvessel. B: Blood vessel. I: Stagnation of Indian ink.

Fig. 7.



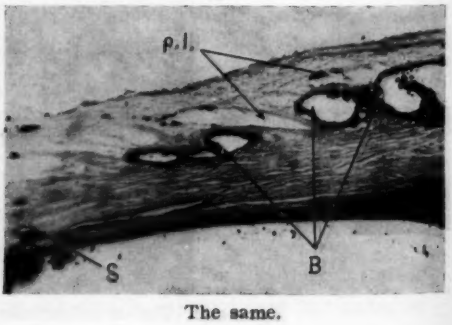
The same. p.l.: Perivascular lymphspace.

Fig. 8.



The same. S: Vessel corresponding to Schlemm's canal.

Fig. 9.



The same.

the fibers and the outer layer in the ciliary body in contact with the sclera. This reaction is still more slight than the one in the iris. In the anterior half of the choroid, there exists sometimes only a little reaction, which is never seen in the posterior half. Throughout all specimens, intrascleral vascular meshwork has a strong ferric reaction in every vessel wall. It was found, that the Berlin blue particles are not only taken up by the endothel cell, but also permeated into the vessel walls with the deposition in the vessel in extreme cases. Most of these vessels are situated in the subconjunctival or episcleral layer but some of them are observed in the deeper layer. These vessels are connected with those which penetrate the sclera running through Fontana's space and pectinate ligament (Fig. 6, 7, 8 and 9).

These findings have led to the idea, that the aqueous humor runs through the corneoscleral trabeculum to pectinate ligament, and is gradually absorbed into blood vessels reaching to episcleral meshwork.

It is noted, that there are several other cases; e.g. 1. relatively strong ferric reaction in the anterior ciliary vein at the insert point of the rectus muscle, 2. slight reaction in the anterior side of the iris and ciliary body and 3. very slight diffuse reaction in the choroid. These facts suggest, that there exists another passage leading from the iris and ciliary body to the vortex vein in addition to the above mentioned way which seems to play the most important role in connecting the aqueous humor with blood vessels.

Discussion

1. Conjunctival lymphvessels.

The binocular corneal microscopic observation in the case of using brilliant cresyl blue eye drops indicates, that there exist several kinds of conjunctival lymphvessels; e.g. 1. those which run along with the superficial blood vessels (so-called "perivascular lymph space") and 2. those which are still more deeply situated, greater than a blood vessel and run independently, and further observation using Indian ink injection shows 3. the most deep lymphvessel meshwork running quite independently from the blood vessels. Furthermore, the histological observations point out that these lymphvessels are capillaries made of one layer of the endothelium with no muscular cells.

2. Aqueous passages.

The main passage is considered to be the following system; i.e. anterior chamber angle—sclerocorneal trabeculum—Fontana's space—vessels corresponding to Schlemm's canal—scleral vascular meshwork. It has been generally assumed, that there exist such normal aqueous passages, that the anterior ciliary vein system runs through the anterior chamber angle to Schlemm's canal and the vortex vein system goes through the iris and the ciliary body. As regards this problem, Dr. Kishimoto has ascertained the way of running from the anterior chamber angle through Schlemm's canal to the surface veins of the eyeball. This judgment can be supported by the

result of this experiment.

I. Asayama (Indian ink case) and Akagi and others (patent blue case) have reported, that stains injected into the anterior chamber of the rabbit's eye are taken into the vortex vein, and Carbella and Gifford have found out most of the stain particles in Fontana's space, and the rest in the choroid and vortex veins through their experiments with tissue specimens. These observations suggest another passage; i.e. the anterior chamber angle—outer layer of the ciliary body—choroid vessel—vortex vein.

Oohashi, Saito and others have discussed that the system, i.e. iridociliary vessels—scleral ciliary veins vortex veins, play an important role in an aqueous passage, and that the passage from the aqueous vein through Schlemm's canal to the anterior ciliary vein is a secondary control system which is usually inactive. The result of this experiment shows, however, that the secondary system, as they call it, is considered as the main aqueous passage, in case the degree of ferric reaction represents a good criterion of aqueous outflow.

3. Relation between conjunctival lymphvessels and aqueous passage (so-called intraocular lymph streams)

Conjunctival lymph streams are considered to run from tissue space to the perivascular lymph spaces and the proper lymphvessels of the conjunctiva without entering into blood streams. It is noted, however, that the origin of the conjunctival lymph streams is not identified.

On the other hand the aqueous fluid which penetrates into tissue spaces from the anterior chamber angle runs not into lymphvessels but into blood streams, which are connected to the surface venous systems of the eyeball. This conclusion is definitely confirmed by the fact, that the ferric ions and trypan blue injected into the anterior chamber were taken into surface venous systems of the eyeball, but not into the lymphvessels, which have been shown by use of the Indian ink injection. In other words the ferric ions injected into the anterior chamber of the rabbit's eye run from the anterior chamber angle through Fontana's space and vesselcorresponding to Schlemm's canal, to scleral venous system without having influence on the lymphvessels and the perivascular lymph spaces. Considering, that the Indian ink particles have an ability to absorb the stain particles, the following experiment was conducted in order to solve the question, whether the absence of the stain particles in lymphvessels was due to the absorption of Indian ink particles or not. After ferric ions were injected into the anterior chamber the eyeball was extirpated followed by the Indian ink injection into the surface blood vessels to observe ferric and Indian ink particles in the vessels. The vessels had ferric as well as Indian ink particles with no finding, indicating the absorption of the former by the latter. The quantity of the stain and the size of the Indian ink particles deposited in the vessels also denied, that the absence of the former in the lymphvessels was due to the absorption of the latter.

It was concluded, that there was a stream corresponding to Schlemm's canal between the Fontana's space and the anterior ciliary vein, and that this stream belonged not to the lymphatic but to the venous system.

As already pointed out, there was no direct communication between conjunctival lymph streams and the aqueous passage. The mechanism which keeps these two streams away from each other was considered to be the difference in the origin of these streams, diffusion due to different contents between lymph and blood, permeability of the vessel walls and different osmotic pressure among tissue fluid, lymph and blood, etc. A part of the aqueous humor (so-called intraocular lymph) may join into the lymph stream but so for there is no definite evidence.

Summary

This paper presents vital and histological examinations on the relation between conjunctival lymphvessels and the aqueous passage (so-called intraocular lymph passage).

1. The application of a brilliant cresyl blue solution eye drops reveals superficial perivascular lymph spaces and lymphatic meshwork independent from blood vessels and a more deeply situated lymphatic meshwork was found by stick-injecting Indian ink. Conjunctival lymph streams run from the tissue space a part to perivascular lymph spaces and the rest to proper lymphvessels.

2. The anterior passage of the aqueous humor is as follows; anterior chamber angle—sclerocorneal trabeculum—Fontana's space—vessel corresponding to Schlemm's canal—anterior ciliary veins of the conjunctiva and sclera. It has been observed, that the aqueous humor runs into every anterior ciliary vein.

3. Aqueous humor penetrating into tissue spaces of the anterior chamber angle runs into the venous system of the ocular surface, and conjunctival lymph streams, into proper conjunctival lymphvessels. There is no direct communication between the two.

4. The vessel corresponding to Schlemm's canal in the rabbit's eye is considered a venous system.

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STUDIES ON THE DEPTH OF THE ANTERIOR CHAMBER

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In the studies of the etiology of glaucoma, the fact seems very interesting that, among the morbid eyes with changes in the depth of the anterior chamber, the depth of the glaucomatous eyes is usually shallow and the shallowness of the anterior chamber is one of the significant symptoms of glaucoma.

Recently, the author measured the depth of the anterior chamber of normal eyes and glaucomatous eyes, and studied the results of the measurement.

The author reports about that.

I. The Axial Depth of the Anterior Chamber in the Normal Eyes

Methods

The apparatus for examination was the Littmann's slit-lamp microscope of Zeiss-Opton's company with the plano-parallel plate of Jaeger (1952).

The author measured the true length between the apex of the anterior surface of the cornea and the anterior surface of the lens. The angle between the optical axis of the measured eyes and the observational axis of the apparatus was settled at 40°. The examined eyes had to be accomodation-free, and the measurement was carried out for 5 times or more on each eye, and then the mean value was calculated.

Results

The author examined 451 normal eyes of 234 persons aged from 8 to 77 years and their refractions were distributed from −1.0 D. to +1.0 D.

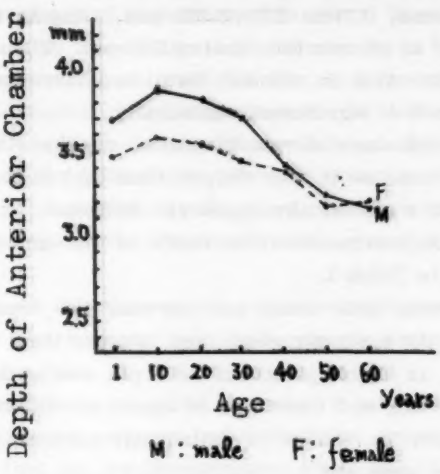
The axial depth of the anterior chamber of the normal eyes changed considerably case by case, and distributed from 2.6 mm to 4.4 mm. The most concentrated distribution was 3.5 mm (12.2%) and the distribution was considerably reduced in the regions of the depth over 3.9 mm and under 3.3 mm. The average depth was 3.54± 0.016 mm. Both eyes of one person were almost always equal in depth of anterior chamber.

The depth of the anterior chamber varied in ages, that is, in early adult age (16–30 years of age) it was the deepest (3.71 mm±0.023 mm) and in school-age (8–15 years) it was 3.66±0.031 mm deep, then in late adult age (31–50 years) it was 3.51± 0.025 mm, and in old age (over 51 years) it was the shallowest (3.18±0.030 mm).

The depth of the anterior chamber seemed to change parallel with the physical growth and degeneration.

The difference of the depth of the anterior chamber between male and female was, that in the growing period, the male had a deeper anterior chamber than the

Fig. 1. Relation curve between the depth of the anterior chamber and the age.



female, but with the over fourties both had equal depth (Fig. 1).

II. The Axial Depth of the Anterior Chamber in the Glaucomatous Eyes

The examining method was the same as that in the normal eyes and the apparatus of Jaeger (1952) was also used.

Results

The author examined 107 eyes with primary glaucoma of 75 persons aged from 32 to 82 years and also 27 eyes with secondary glaucoma of 24 persons aged from 10 to 68. Among the 107 eyes with primary glaucoma, there were 31 eyes with acute congestive glaucoma of 25 persons, them, 53 eyes with chronic congestive glaucoma of 34 persons, and 23 eyes with simple glaucoma of 16 persons. Among them, all the congestive glaucomas, both acute and chronic, belonged to the narrow-angle glaucoma and, in contrast with them, all the simple glaucomas belonged to the wide-angle glaucoma.

1. The axial depth of the anterior chamber in the eyes with primary glaucoma.

The axial depth of the anterior chamber was various in the glaucomatous eyes as that was in normal eyes.

In the eyes with congestive glaucoma, it was distributed from 1.4 to 2.9 mm, and among them, in acute form the depth was distributed 1.4-2.8 mm, and in chronic form 1.8-2.9 mm. So, it was very shallow as compared with that in normal eyes.

In the eyes with simple glaucoma, on the other hand, the depth was distributed from 2.7 mm to 3.6 mm and it was a little shallower than the depth in normal eyes, but was deeper than that in congestive glaucomatous eyes.

In all the eyes with primary glaucoma, the average depth of the anterior chamber was 2.48 ± 0.041 mm deep.

Then, the average depth in every kind of glaucoma was investigated. In narrow-angle congestive glaucoma, it was 2.33±0.035 mm. Among them, in acute form it was 2.13±0.055 mm and in chronic form 2.44±0.058 mm. Then, the depth was shallower in acute form than that in chronic form, and there was significant difference between them in 5% level of significance statistically.

In the eyes with wide-angle simple glaucoma, on the other hand, the average depth was 3.04±0.058 mm, so it was deeper than that in congestive glaucoma, and between them there was a statistically significant difference.

The results of the measurement of the depth of the anterior chamber in every age group were shown in Table 1.

In congestive glaucoma, both acute and chronic, the depth was shallower than that in normal eyes in the same age group, and between them there was a significant difference. In contrast, in simple glaucoma the depth was as deep as that in normal eyes in the same age group, and there was no significant difference between them.

2. The relations between the change of intraocular pressure and the depth of the anterior chamber in the same eye.

The author performed the following experiments to confirm, whether the depth of the anterior chamber of the eyes with primary glaucoma was influenced and made shallower by the increase of the intraocular pressure. For the experiments the author chose the eyes which had narrow-angles and seemed to have a disposition to acute glaucoma, that is, the eyes which had histories seemed to be the prodrome of the glaucomatous attack, or the fellow eyes of which had the attack of acute glaucoma.

At first, the author measured the depth of the anterior chamber of these eyes in normal intraocular pressure, then provocated the elevation of the intraocular pressure

Table 1. Average in Different Age Groups of the Axial Depth of the Anterior Chamber of Primary Glaucoma and Normal Eyes (mm)

	30—39 Years		40—49 Years		50—59 Years		60 Years and Older		Total			
	Eyes	M±m	Eyes	M±m	Eyes	M±m	Eyes	M±m	Eres	M±m	Eyes	M±m
Normal eyes	62	3.61 ±0.038	63	3.44 ±0.031	66	3.21 ±0.035	33	3.17 ±0.051	224	3.38 ±0.022		
Congestive glaucoma (acut and chronic)			9	2.39 ±0.092	25	2.46 ±0.059	50	2.25 ±0.045	84	2.33 ±0.035		
Acute congestive glaucoma					5	2.06 ±0.147	26	2.14 ±0.060	31	2.13 ±0.055		
Chronic congestive glaucoma			9	2.39 ±0.092	20	2.57 ±0.041	24	2.36 ±0.058	53	2.44 ±0.036	107	2.48 ±0.041
Simple glaucoma	2	3.50 ±0.100	2	3.25 ±0.016	4	2.93 ±0.063	15	2.99 ±0.070	23	3.04 ±0.058		

M: Average of the depth of the anterior chamber
m; Standered error

by the darkroom experiment (7 eyes) or by the mydriasis test by neosynesin (4 eyes) and again measured the depth of the anterior chamber. Next, the author decreased the intraocular pressure by the miotica and Diamox to be of normal pressure, and measured again the depth of the anterior chamber, in a few days since that, not any drag was used. As the results of the experiments, the author recognized, that the depth of the anterior chamber did not change beyond the range of observational errors at any phase of the intraocular pressure variation, that is, the depth of the anterior chamber was not influenced by the change of the intraocular pressure.

3. The axial depth of the anterior chamber in both eyes of the patient with one-sided primary glaucoma.

The comparison between the glaucomatous eye and non-glaucomatous eye. The author compared both eyes of the patients, who had primary glaucoma in one eye and had no glaucoma in the other eye.

Among the 20 patients with oue-sided glaucoma, 9 patients had an equal depth in both eyes, but the other 11 patients had a shallower anterior chamber in the glaucomatous eyes, than that in non-glaucomatous eyes, and the maximum difference between both eyes was 0.8 mm. The average depth of the anterior chamber of the eyes with glaucoma was 2.24±0.067 mm and that of the eyes without glaucoma was 2.41±0.043 mm. Then, the average depth with glaucoma was shallower than without glaucoma, and there was a significant difference between them in 5% level of significance statistically. As the results of the observations for one year on the depth of the anterior chamber in one-sided glaucoma, the author noticed that almost always, the depth did not change and the difference of the depth between both eyes was not influenced by the duration of glaucoma, that is, there were such cases that one had glaucoma for a year and did not have a difference of depth between both eyes, and, on the contrary, the other had glaucoma for only a short time and had a considerably large difference between both eyes. Then, it seemed that one could hardly say, that the depth of the anterior chamber became shallower with the duration of glaucoma.

Table 2. Relations between Normal Eyes and Non-Glaucomatous Eyes with One-Sided Primary Glaucoma (mm)

	30～39 Years		40～49 Years		50～59 Years		60 Years and Older		Total	
	Eyes	M±m	Eyes	M±m	Eyes	M±m	Eyes	M±m	Eyes	M±m
Normal eyes	62	3.61 ±0.038	63	3.44 ±0.031	66	3.21 ±0.035	33	3.17 ±0.051	224	3.38 ±0.022
Non-glaucomatous eyes in one-sided congestive glaucoma			3	2.53 ±0.121	4	2.45 ±0.155	13	2.37 ±0.040	20	2.41 ±0.043
Non-glaucomatous eyes in one-sided simple glaucoma	2	3.55 ±0.016					5	3.06 ±0.147	7	3.20 ±0.136

M: Average of the depth of the anterior chamber

m: Standerd error

Among the 7 patients with one-sided simple glaucoma, 5 patients had no difference of depth between both eyes, and the other two had but slight differences. The glaucomatous eyes were shallower than the other by 0.1 mm. The average depth of the anterior chamber of glaucomatous eyes in these patients was 3.17±0.131 mm, and that of non-glaucomatous eyes was 3.20±0.136 mm. The former was scarcely shallower than the latter, but there was no significant difference statistically.

The comparison between the normal eyes and the non-glaucomatous eyes of the patients with one-sided glaucoma (*Table 2*). In all the non-glaucomatous eyes of the patients with one-sided congestive glaucoma, the depth of the anterior chamber was shallow in comparison with the average depth of the normal eyes in the same age group, and the statistically significant difference was recognized between them in every age group. But, between the non-glaucomatous eyes in one-sided simple glaucoma and the normal eyes in the same age group, no significant difference could be recognized about the depth.

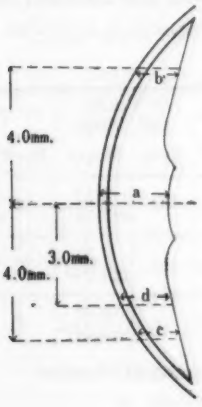
In one-sided congestive glaucoma, the average depth of non-glaucomatous eyes was shallow in comparison with that of non-glaucomatous eyes in one-sided simple glaucoma, and between them there was a significant difference statistically.

4. The axial depth of the anterior chamber in secondary glaucoma.

In 27 eyes with secondary glaucoma of 24 patients, the average depth of the anterior chamber was 3.12±0.115 mm. In these cases, the depth was influenced by the causative diseases, and varied irregularly case by case. So, in these cases, the studies about the difference of the depth of the anterior chamber were not interesting and were meaningless on the inquiry of glaucoma proper.

III. Relations between the Peripheral and the Axial Depth of the Anterior Chamber in the Glaucomatous Eyes

Fig. 2. Measured positions of the depth of the anterior chamber.



Methods

The author took the slit photograph of the anterior part of the eyes by the Littmann's slit-lamp microscope of Zeiss-Opton's company with a photographic attachment.

The author set the apparatus to the angle for observations (45°) and took the photographs 3 times in each eye with the size of the original, then calculated the mean value of them. The methods of measurement of the depth of the anterior chamber was as follows :

The slit-photograph taken by the apparatus was projected in accurately tenfold enlargement and the author measured the depth on the projected picture, then calculated the ten-division of the measured value. The 4 points, where the depth of anterior chamber was measured, were illustrated in Fig. 2. The point "a" illustrates the axial depth, that is, the depth from the apex of the cornea to the anterior surface of the lens. Point "b" illustrates the peripheral depth, 4.0 mm upward from the visual axis. Point "c" illustrates also the peripheral depth, 4.0 mm below the axis. Point "d" illustrates the peripheral depth 3.0 mm below the axis. The author calculated the ratios between peripheral and axial depth: b/a, c/a and d/a. The small ratios mean the high degree of the iris bombé. The values measured in the examination were of course the observational depths at the angle of 45, but not the true depth.

Results

1. Relations between the upper peripheral depth and the lower peripheral depth of the anterior chamber.

In 30 normal eyes of 15 persons, the average value of the iris bombé in the upper periphery (b/a) was 0.58 ± 0.006, and that in lower periphery (c/a) was 0.62 ± 0.007. Then, the former is smaller than the latter at a 5% level of significance statistically.

In 49 eyes of 39 patients with narrow-angle primary congestive glaucoma (in preoperational time) the mean value of the ratio b/a, 0.46 ± 0.007, was smaller than that of the ratio c/a, 0.50 ± 0.008, and a significant difference was recognized. Then, it might be agreeable that the degree of iris bombé was higher in the upper part of the iris than that in the lower part.

Next, in comparison of the ratios b/a, c/a, between the eyes with primary congestive glaucoma and the normal eyes, the ratios were smaller in the former than that in the latter, and a significant difference was recognized. Then, it was also agreeable that the degree of iris bombé was higher in glaucomatous eyes than that in normal eyes.

2. The depth of the anterior chamber of the eyes with primary congestive glaucoma before and after operation.

a. The axial depth of the anterior chamber (Table 3).

In 30 eyes of 25 patients with narrow-angle primary congestive glaucoma, the author measured the depth of the anterior chamber by the apparatus of Jaeger before and after operation, and compared the depth with each other. The cases examined were all successfully operated on. The author measured the true length from the apex of the cornea to the anterior surface of the lens.

The measured value of the depth are illustrated in Table 3. The average difference of the depth of the anterior chamber between the times before and after operation was very small, 0.003 mm, and in cases the depth in the time after opera-

Table 3. Aixal Depth of the Anterior Chamber in the Primary Congestive Glaucomatous Eyes before and after Operation (by apparatus of Jeager)

Eye No.	Age	Sex	Side	Kinds	Depth of Anterior Chamber (mm)			Methods of Operation	Duration between the op. and the measurement after it (days)
					Before Op. (Ⅰ)	After Op. (Ⅱ)	Difference (Ⅰ)—(Ⅱ)		
1	40	M	L	C.C.G.	2.7	2.7	0	Peripheral Iridencleisis	22
2	40	M	L	〃	1.8	1.8	0	〃	33
3	41	F	R	〃	2.5	2.6	0.1	〃	59
4	41	F	L	〃	2.5	2.6	0.1	〃	46
5	41	F	R	〃	2.5	2.6	0.1	Peripheral Iridectomy	33
6	41	F	L	〃	2.5	2.5	0	Peripheral Iridencleisis	47
10	52	F	L	〃	2.3	2.3	0	Trephining	53
12	54	F	L	〃	2.6	2.6	0	Peripheral Iridencleisis	25
13	55	F	L	〃	2.8	2.8	0	〃	112
16	57	M	L	〃	2.8	2.7	—0.1	〃	26
19	57	F	R	A.C.G.	2.6	2.6	0	Peripheral Iridectomy	23
22	59	F	R	C.C.G.	2.6	2.7	0.1	〃	21
23	59	F	L	〃	2.6	2.6	0	〃	20
24	60	F	R	〃	2.9	2.9	0	Trephining	21
25	61	M	R	〃	2.7	2.6	—0.1	Peripheral Iridencleisis	77
27	61	F	R	〃	2.1	2.2	0.1	Peripheral Iridectomy	118
28	61	F	R	A.C.G.	2.1	1.8	—0.3	〃	51
29	61	F	L	〃	2.2	2.1	—0.1	〃	41
31	63	F	R	C.C.G.	2.1	2.2	0.1	〃	24
33	64	F	L	A.C.G.	2.4	2.2	—0.2	Peripheral Iridencleisis	28
35	66	M	R	C.C.G.	2.2	2.2	0	Peripheral Iridectomy	83
37	66	F	R	A.C.G.	2.2	2.3	0.1	〃	21
38	66	F	L	〃	2.1	2.2	0.1	〃	30
39	67	F	L	〃	2.1	2.3	0.2	〃	31
40	68	F	L	〃	2.6	2.7	0.1	〃	18
43	70	M	R	C.C.G.	2.0	1.8	—0.2	Peripheral Iridencleisis	38
45	74	F	L	A.C.G.	1.8	1.8	0	〃	59
46	74	F	R	〃	2.3	2.2	—0.1	Peripheral Iridectomy	83
47	75	F	R	〃	1.4	1.5	0.1	〃	17
48	77	F	R	C.C.G.	2.7	2.7	0	Peripheral Iridencleisis	46
					Average of the Difference		0.003		

A.C.G.: Acute congestive glaucoma
C.C.G.: Chronic congestive glaucoma

tion was deeper than that before operation, but in the others the depth after operation was shallower. Statistically, a significant difference was not recognized between the depth in two times.

b. The peripheral depth of the anterior chamber.

Peripheral Iridectomy (*Table 4*): In 15 eyes of 12 patients with narrow-angle primary congestive glaucoma, the author measured the degree of iris bombé in the lower part

Table 4. Peripheral Iris Bombé of the Lower Part in Primary Narrow-angle Glaucomatous Eyes before and after Peripheral Iridectomy

Eye No.	Age	Sex	Side	c/a			d/a			Angle of the anterior chamber after the operation
				Before Op. (I)	After Op. (II)	Difference (II)—(I)	Before Op. (I)	After Op. (II)	Difference (II)—(I)	
5	41	F	R	0.45	0.54	0.09	0.57	0.64	0.07	1/2 round open
19	57	F	R	0.41	0.47	0.06	0.58	0.61	0.03	partially open
22	59	F	R	0.50	0.56	0.06	0.66	0.70	0.04	1/2 round open
23	59	F	L	0.52	0.59	0.07	0.67	0.73	0.06	all round open
27	61	F	R	0.48	0.57	0.09	0.55	0.70	0.15	all round open
28	61	F	R	0.53	0.59	0.06	0.68	0.72	0.04	partially open
29	61	F	L	0.44	0.58	0.14	0.58	0.69	0.11	all round open
31	63	F	R	0.40	0.62	0.22	0.52	0.66	0.14	all round open
35	66	M	R	0.56	0.74	0.18	0.58	0.82	0.24	all round open
37	66	F	R	0.44	0.57	0.13	0.59	0.67	0.08	all round open
38	66	F	L	0.47	0.72	0.25	0.59	0.80	0.21	all round open
39	67	F	L	0.48	0.63	0.15	0.95	0.98	0.03	1/2 round open
40	68	F	L	0.48	0.52	0.04	0.63	0.66	0.03	partially open
46	74	F	R	0.57	0.69	0.12	0.73	0.76	0.03	all round open
47	75	F	R	0.51	0.64	0.13	0.69	0.78	0.09	unknown
				Average of the Difference		0.12	Average of the Difference		0.09	

Table 5. Peripheral Iris Bombé of the Lower Part in Primary Narrow-angle Glaucomatous Eyes before and after Peripheral Iridencleisis

Eye No.	Age	Sex	Side	c/a			d/a			Angle of the anterior chamber after the operation
				Before Op. (I)	After Op. (II)	Difference (II)—(I)	Before Op. (I)	After Op. (II)	Difference (II)—(I)	
1	40	M	L	0.54	0.56	0.02	0.68	0.69	0.01	all round close
2	40	M	L	0.56	0.68	0.12	0.67	0.69	0.02	partially open
3	41	F	R	0.51	0.53	0.02	0.63	0.67	0.04	partially open
4	41	F	L	0.50	0.53	0.03	0.62	0.66	0.04	1/2 round open
6	41	F	L	0.46	0.62	0.16	0.62	0.72	0.10	1/2 round open
12	54	F	L	0.52	0.59	0.07	0.65	0.68	0.03	1/2 round open
13	55	F	L	0.46	0.48	0.02	0.61	0.63	0.02	unknown
16	57	M	L	0.58	0.60	0.02	0.71	0.74	0.03	partially open
25	61	M	R	0.46	0.63	0.17	0.59	0.71	0.12	1/2 round open
33	64	F	L	0.63	0.69	0.06	0.72	0.79	0.07	3/4 round open
43	70	M	R	0.51	0.60	0.09	0.57	0.67	0.10	1/2 round open
45	74	F	L	0.59	0.60	0.01	0.66	0.66	0	unknown
48	77	F	R	0.45	0.47	0.02	0.82	0.83	0.01	all round close
				Average of the Difference		0.06	Average of the Difference		0.05	

of the iris, c/a and d/a, in the times before and after the peripheral iridectomy. The author recognized, that the ratios, c/a and d/a, were increased after the operation in all cases, that is, the difference of the ratio, c/a, was 0.12 and that of d/a was 0.09,

then, a statistically significant difference was recognized.

After the operation, in the angle of the anterior chamber the iris bombé considerably reduced in all cases, and the angle was considerably wide in its part without adhesion, so, in most cases the trabeculae and also a part of ciliary body were visible, but the degree of improvment was varied case by case.

The extent of the opening of the angle by operation was conditioned by the degree of the adhesion in the angle, and among the examined cases, in 8 eyes (53.33%) the angle was entirely opened all around, in 3 eyes (20%) it was opened in half circumference, in 3 cases (20%) it was opened only partially and in one case (6.67%) the results were not known. The demonstrative cases were shown in Fig. 3 (the eye No. 31) and in Fig. 4 (the eye No. 38).

Peripheral Iridencleisis (*Table 5*): In 13 eyes of 12 patients, the ratio c/a was increased after operation, and the ratio d/a was increased in 12 eyes but did not change in one. In comparison with the cases of peripheral iridectomy, however, the differences of the ratios were small. The average difference of the ratio c/a was 0.06 and that of the ratio d/a was 0.05. Statistically, however, there was a significant difference between the ratios, c/a and d/a, before the operation and those after the operation.

After the operation, the angle in these cases had the same figure as the angle in the eyes with peripheral iridectomy. But, before operation in these cases, the degree of the organized adhesions at the angle was more intense than that in the cases

Fig. 3. a. Eye No. 31.
Before Op.

Fig. 3. b. Eye No. 31
After Peripheral Iridectomy.

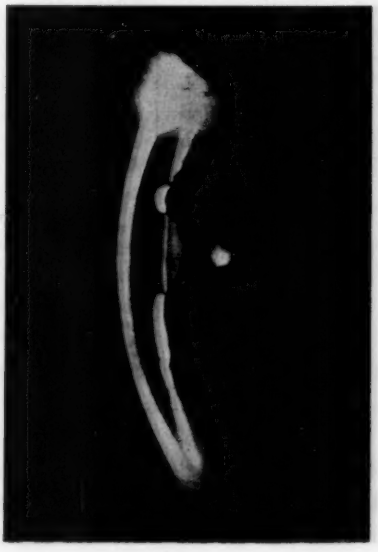


Fig. 4. a. Eye No. 38
Before Op.

Fig. 4. b. Eye No. 38
After Peripheral Iridectomy.

Table 6. Peripheral Iris Bombé of the Lower Part in Primary Narrow-angle Glaucomatous Eyes before and after Trephining

Eye No.	Age	Sex	Side	c/a			d/a			Angle of the anterior chamber after the operation
				Before Op. (Ⅰ)	After Op. (Ⅱ)	Difference (Ⅱ)—(Ⅰ)	Before Op. (Ⅰ)	After Op. (Ⅱ)	Difference (Ⅱ)—(Ⅰ)	
10	52	F	L	0.49	0.50	0.01	0.67	0.62	0	all round close
24	60	F	R	0.58	0.60	0.02	0.70	0.67	—0.03	all round close
				Average of the Difference		0.02	Average of the Difference		—0.02	

of peripheral iridectomy, and more, the adhesion advanced after operation, because the anterior chamber was not formed for few days, then, after operation the extent of the open-angle was not wide, and the angle was observed at a glance as if it had not changed.

Among the examined cases, in no eye the angle was opened entirely, in 1 eye (7.69%) the angle was opened in $^3/_4$ circumference, in 5 cases (38.46%) it was opened in 1/2, in 3 (23.07%) only partially, in 2 (15.39%) it was closed totally and in 2 eyes the results were not known. The degree of iris bombé, however, was decreased, then, the iris became flatter as the iris in the eyes of peripheral iridectomy.

Trephining (*Table 6*): In 2 eyes of 2 patients, the ratios, c/a and d/a were not changed by the operation, and after operation, the angle was entirely closed in both cases.

IV. Relations between the Axial Depth of the Anterior Chamber and the Thickness of the Lens in the Glaucomatous Eyes

Methods

The methods of examination were as follows: The axial depth of the anterior chamber and the observational thickness of the lens were measured by the apparatus of Jaeger (1952). And then, the true thickness of the lens was calculated by the formula of Oshima (1959).

Results

The author examined 104 normal eyes of 57 persons in which the refractions were within ±1.0 D, and 26 eyes of 19 patients with the narrow-angle primary congestive glaucoma.

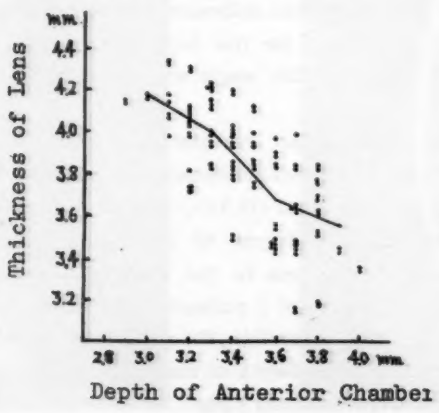
1. The thickness of the lens in normal eyes.

The value of the thickness of the lens in normal eyes distributed from 3.15 mm to 4.33 mm and the mean value was 3.83±0.028 mm. In every age group which was divided into every ten years, the mean thickness of the lens was calculated. The lens was thinnest in the teen-age group and the mean value was 3.42±0.044 mm, next to them it was 3.57±0.047 mm in the twenties, and then 3.65±0.044 mm in the thirties, 3.90±0.017 mm in the forties, 4.07±0.028 mm in the fifties, 4.14±0.035 mm in the sixties and older. The older the age was, the thicker was the lens. The relations between the depth of the anterior chamber and thickness of the lens were illustrated in Fig. 5, and the correlation coefficient was $r \pm Mr = -0.7602 \pm 0.0414$ and the correlation ratio was $\eta = -0.7981$, then this was the inverse correlation. Both in "r" and in "η", a statistically significant difference was recognized.

2. The thickness of the lens in the eyes with primary congestive glaucoma.

In 26 eyes of 19 patients with narrow-angle primary congestive glaucoma, the thickness of the lens was as follows: the mean value of the thickness in the age

Fig. 5. Correlative figure between the depth of the anterior chamber and the thickness of the lens in normal eyes.



group of the fifties was 4.21±0.037 mm, and that in the group over 60 years was 4.34±0.017 mm. In comparison with the normal eyes in the same age group the thickness of the lens in the glaucomatous eyes was thick, and a statistically significant difference was recognized.

Discussion

About the cause of the congestive glaucoma, in the mechanical theory, it was said, that the primary cause of the disease was the anatomical abnormality, that is, the narrowness of the angle of the anterior chamber. Because of that, the angle was closed by chance and then the intraocular pressure rose. Further upon this fundamental abnormality, as the cause increasing the narrowness of the angle and finally closing the angle, Curran (1920), Chandler (1952), Barkan (1954) et al gave their attentions to the physiological iris bombé in the peripheral part of the iris.

Namely, in the eyes with narrow-angle because of the shallowness of the anterior chamber by swelling or by the advancement of the lens, the contact surface of the iris to the anterior surface of the lens was increased, and then the physiological pupillary seclusion was also intensified, so the circulation of the aqueous fluid was disturbed and the pressure of the posterior chamber became higher, than that of the anterior chamber.

Then, the root of the iris was protruded forward and the angle of the anterior chamber was closed.

Therefore, they advocated the peripheral iridectomy, because the excision of the root of the iris excluded the disturbances of the aqueous circulation from the posterior to the anterior chamber, then, the iris bombé was reduced and the angle of the chamber was opened, so, the outflow of the aqueous fluid became free, and the intraocular hypertension was prevented.

The author compaired the axial depth of the anterior chamber of the glaucomatous eyes with that of the normal eyes in the same age group. Among the eyes with primary glaucoma, the eyes with narrow-angle primary congestive glaucoma, both acute and chronic, had a shallower anterior chamber than the normal eyes, and especially in acute congestive glaucoma, the anterior chamber was shallow, but in the wide-angle simple glaucoma, the depth of the anterior chamber was not shallower than that of the normal eyes.

These results of the examination accorded with the reports of Raeder (1923), of Rosengren (1931), of Shindo (1933) and of Törnquist (1956), and by the results it was obvious that the cause of the congestive glaucoma differed from that of the simple glaucoma.

The shallow anterior chamber in the congestive glaucoma did not change its depth by the change of the intraocular pressure, and these findings were in accord with the report of Rosengren (1931).

Further more, the author found, that in the non-glaucomatous eyes of the patients with one-sided congestive glaucoma, the depth was scarcely deeper than that of the other eyes with glaucoma, but was extremely shallower, than that of the normal eyes in the same age group, and that, between both eyes of the one-sided glaucomatous patient, the difference of the depth did not relate to the duration of the disease, and the depth did not become shallower with the duration of the glaucoma.

According to the findings above mentioned, the author considered, that the shallow anterior chamber was not the result of the high intraocular pressure, but it was before the onset of the disease and was the disposition of the disease, and that, the shallower the anterior chamber was, the nearer was the danger of an acute attack of glaucoma, and also that the difference of the depth between both eyes of the patient with one-sided glaucoma was in existence before the onset of glaucoma as the difference of the disposition to the disease.

Then, the author investigated the relation between the axial and the peripheral depth of the anterior chamber of the eyes with narrow-angle congestive glaucoma by slit-photograph, and found the fact, that the peripheral part of the iris protruded more in the upper part than that in the lower part, and this fact was in accord with the reports of Barkan (1936), of Ogino (1950) and of Phillips (1956).

By this examination, the author substantiated also the reports of Barkan (1938), of Phillips (1956) and of Ogino (1959) in which they mentioned, that the peripheral adhesion of the iris in narrow-angle glaucoma occurred mainly in the upper part.

In glaucomatous eyes, in both upper and lower parts, the peripheral iris protruded forward more intensely, than that in normal eyes. Although, in narrow-angle glaucoma, the axial depth of the anterior chamber did not change by operation, in most cases, the angle was opened in a wide range by the flattening of the iris after the peripheral iridectomy.

These facts were also mentioned partly by the report of Törnquist (1959), but the report of these facts with the findings of gonioscopy have not appeared. By the facts above mentioned, the author considered, that the depression of the intraocular pressure by peripheral iridectomy was induced by the opening of the anterior chamber angle, and the opening was induced by the flattening of the iris, and then, it was obvious, that the physiological iris bombé existed really.

And finally, the author observed, that the lens of the eyes with congestive glaucoma was thicker, than that of the normal eyes in many cases, and this fact substantiated the theory of Curran, of Chandler and Barkan.

Summary

The author measured the depth of the anterior chamber and the thickness of the lens of the glaucomatous and the normal eyes by the Littmann's slit-lamp microscope of Zeiss-Opton's company with th plano-parallel plate of Jaeger. Then, the author

took the slit-photograph of the anterior part of the eye by the apparatus with photographic attachment of Zeiss-Opton's company, and calculated the ratio between the axial depth and the peripheral depth of the anterior chamber. The author examined 451 normal eyes of 234 persons, 31 eyes of 25 patients with acute congestive glaucoma, 53 eyes of 34 patients with chronic congestive glaucoma, 23 eyes of 16 patients with simple glaucoma, and 27 eyes of 24 patients with secondary glaucoma.

1. The mean value of the axial depth of the anterior chamber in primary glaucoma was 2.48 ± 0.041 mm. Among them, the mean value of the depth was 2.13 ± 0.055 mm in acute congestive glaucoma, 2.44 ± 0.036 mm in chronic congestive glaucoma, and 3.04 ± 0.058 mm in simple glaucoma. In every age group, the depth of the anterior chamber was shallower in acute and chronic congestive glaucoma than that in normal eyes, but it was not shallower in the eyes with simple glaucoma than that in normal eyes.

2. The depth of the anterior chamber if primary glaucoma did not change by the variation of the intraocular pressure. The depth did not change in most cases in the duration of the disease.

3. In one-sided congestive glaucoma, the glaucomatous eye had generally a shallower anterior chamber, than the other non-glaucomatous eye. But, the latter had also a shallower anterior chamber, than the normal eyes in the same age group.

On the other hand, in one-sided simple glaucoma, the difference between both eyes of one patient was not significant.

4. The average axial depth of the anterior chamber in the secondary glaucoma was 3.12 ± 0.015 mm.

According to the findings above mentioned, the author considered, that shallowness of the anterior chamber was not the result of the high intraocular pressure, but it existed before the onset of congestive glaucoma and it was the result of the disposition of this disease.

5. In both normal and glaucomatous eyes, the degree of iris bombé was more intense on the upper part of the iris, than that which was on the lower part of the iris.

The iris bombé was more distinguished in the eyes with primary congestive glaucoma, than it was in normal eyes.

The axial depth of the anterior chamber in narrow-angle congestive glaucoma did not change in most cases by the operation. But the iris bombé was markedly reduced after the peripheral iridectomy and the iris became flat, and then, the angle of the chamber was opened.

Then, the author considered, that at the presence of the physiological iris bombé it was impossible to deny this as a cause of the increase of the intraocular pressure.

6. In primary congestive glaucoma the lens was thicker, than that which was in normal eyes of the same age group.

By the findings above mentioned, the author accepted the theory, that the cause

of the congestive glaucoma was the high intraocular pressure which was provoked by the following mechanism.

The increased thickness of the lens lead to the shallowness of the anterior chamber, and the shallow anterior chamber and the physiological iris bombé caused the narrowness or the closure of the angle of the anterior chamber.

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STUDIES ON THE ORBITAL CIRCULATION

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I. SIGNIFICANCE OF ORBITAL VESSELS TO OCULAR BALLISTIC MOVEMENT

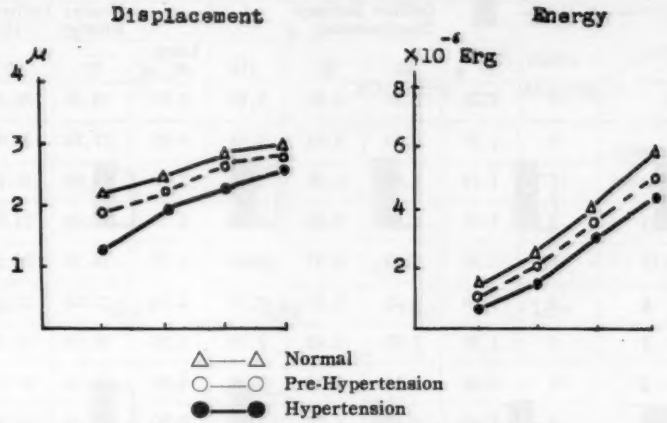
On the previous report, the author published the result of experiment, in which, the recording of ocular ballistic movement under varions loading to the eye ball were made and the forward motion energy of the eye ball was estimated from the value of ocular antero-posterior displacement and time (Fig. 1). The displacement showed linear correlation to the energy within a limited range and was also correlated to the heart stroke volume.

In this series of experiment the relation between ocular ballistic movement and the condition of retrobulbar arteries in the orbita are investigated. And also the medium is provisionally assumed as homogenous, and the following results were obtained.

Method

As stated in the previous repost, ocular ballistic movement was recorded in step-up loading (4 ranges) during 8 seconds utilizing an Ocular-Ballistometer. In

Fig. 1. Variance of ocular ballistic movement.



some of the cases electrocardiogram was taken simultaneously.

From the recorded ocular ballistic movement-curve ocular displacement was estimated. The displacement value was converted to μ unit by comparing it with calibrating volt recorded simultaneously, and there from motorical energy was computed by $^1/_2\ mv^2$ formula.

Sodeman's formula was used for the computation of heart stroke volume except in the cases, in which the obtained value differs much from the value calculate by ballistocardiography or Aizawa's acetylen method. Sodeman's formula would not be applicable in some of the hypertensive cases.

Object of Experiment

The age of the patient ranges from 19 to 63. They include 18 normal persons (average age 33.9) as a control group, 17 patients with essential hypertension (average age 47.8), and 8 patients with labile blood pressure (so called pre-hypertensive stage by Fishberg).

Results

1. Displacement and kinetic energy of the ocular ballistic movement by antero-posterior movement: Fig. 1 shows their change as a consequense of step-up loading in the antero-posterior direction. The control group showed approximately the same result to the previous report. Displacement and energy showed parallel increase with loading step-ups. Hypertension and pre-hypertensive groups showed similar results, except, that their displacement and energy value and their increase due to step-up loading were markedly lower, than that of normal group (Table 1 and Fig. 2).

2. Displacement and energy of antero-posteriorly loaded ocular ballistic movement in the hypertensive group.

Table. 1.

		Total Cases	Ocular Ballistic Displacement μ				Ocular Ballistic Energy ×10⁻⁶Erg			
			Load 35 g	65	90	115	Load 35 g	65	90	115
Normal		18	1.99	2.31	2.62	2.99	8.94	23.89	35.35	58.58
Pre-Hypertension		8	1.80	2.14	2.64	2.78	8.05	17.78	33.88	48.71
Hypertension		17	1.19	1.99	2.32	2.72	5.29	14.66	29.66	42.68
Keith-Wagener	I	4	1.70	1.85	2.20	2.48	5.75	16.20	24.45	51.97
	II	9	1.90	2.10	2.31	2.82	5.12	14.20	26.96	40.77
	III	4	1.58	1.90	2.17	2.78	3.52	11.05	22.00	53.18
Sheie	I	5	1.80	2.00	2.40	2.70	5.26	16.04	26.22	51.97
	II	10	1.84	2.11	2.29	2.76	5.02	14.45	25.74	43.29
	III	2	1.40	1.40	1.75	2.65	3.00	6.10	14.80	23.80

Fig. 2. Relationship between ocular ballistic movement and energy of normal and hypertensive patient.

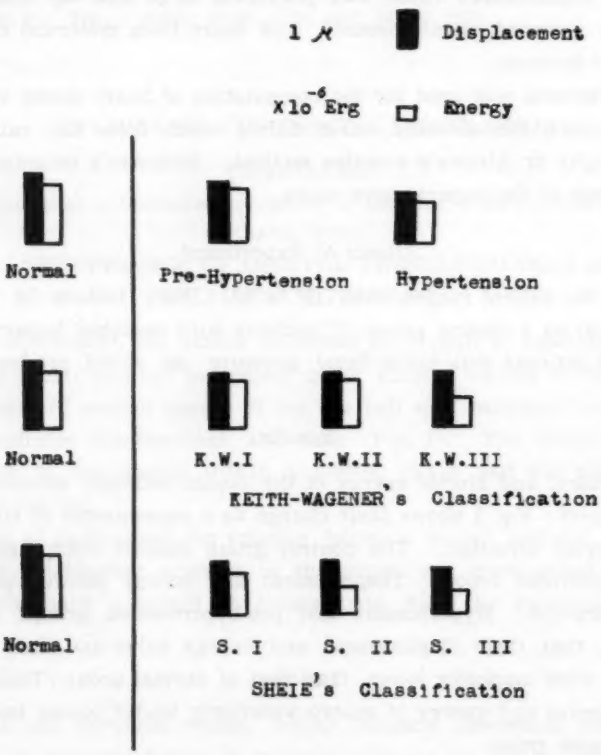


Fig. 3. Relation between blood pressure and ocular ballistic movement.

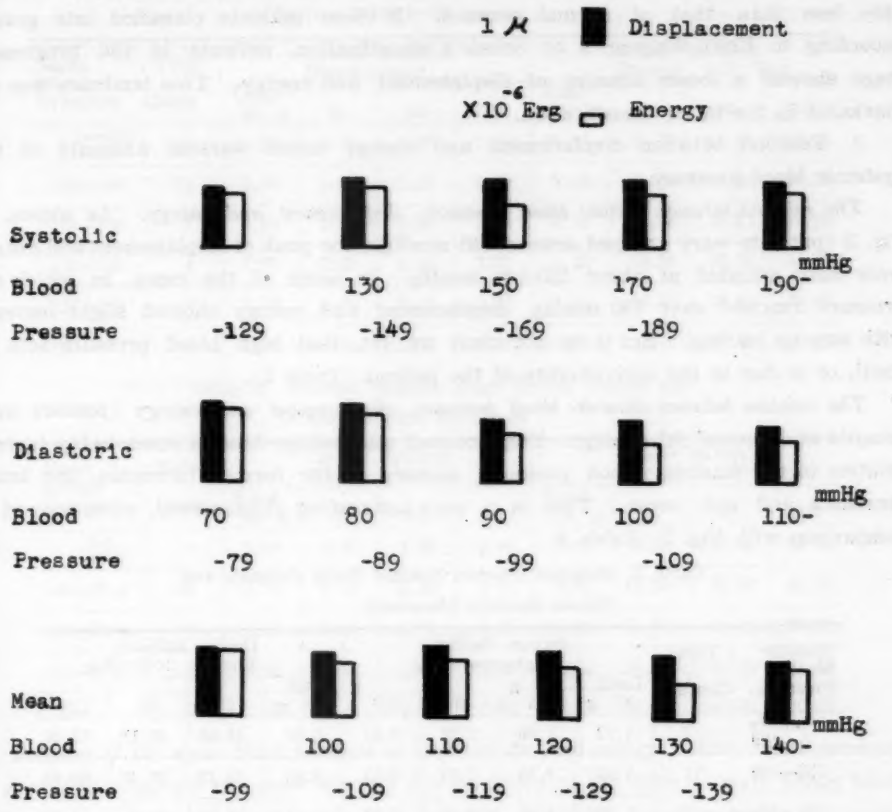
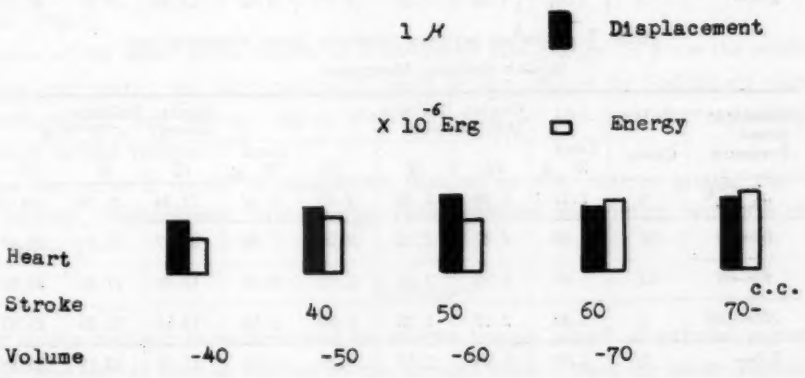


Fig. 4. Relation between heart stroke volume and ocular ballistic movement.



As illustrated in Fig. 1 the displacement and energy of hypertension were remarkably less than that of normal persons. If these patients classified into groups according to Keith-Wagener's or Scheie's classification, patients in the progressed stage showed a lesser amount of displacement and energy. This tendency was remarkable in the latter classification.

3. Relation between displacement and energy under various amounts of the systemic blood pressure.

The relation between systolic blood pressure, displacement and energy. As shown in Fig. 3 (patients were grouped around 150 mmHg), the peak of displacement and energy were both situated at about 130-140 mmHg. In some of the cases, in which the pressure reached over 190 mmHg, displacement and energy showed slight increase with step-up loading. But it is not clear as yet, that high blood pressure acts by itself, or is due to the individuality of the patient (Table 2).

The relation between diastolic blood pressure, displacement and energy (patients were grouped in 5 around 90 mmHg). Displacement and energy have a remarkably inverse relation to the diastolic blood pressure, namely if the former increases, the latter decreases and vice versa. This is a very interesting phenomenon, when viewed in comparison with Fig. 3 (Table 3).

Table. 2. Relation between Systolic Blood Pressure and Ocular Ballistic Movement

Systolic Blood Pressure	Total Cases	Ocular Ballistic Displacement μ				Ocular Ballistic Energy ×10⁻⁶Erg			
		Load 35 g	65 g	90 g	115 g	Load 35 g	65	90	115
mmHg ~129	7	1.74	1.96	2.27	2.61	6.86	16.86	27.13	53.36
130~149	17	1.92	2.36	2.83	3.09	8.88	22.22	36.46	60.45
150~169	13	1.80	1.98	2.30	2.66	5.11	14.34	34.50	43.37
170~189	2	2.10	2.35	2.45	3.05	4.65	13.00	3.75	42.15
190~	3	1.60	1.93	2.50	2.77	4.63	13.30	37.57	46.43

Table. 3. Relation between Diastolic Blood Pressure and Ocular Ballistic Movement

Diastoric Blood Pressure	Total Cases	Ocular Ballistic Displacement μ				Ocular Ballistic Energy ×10⁻⁶Erg			
		Load 35 g	65	90	115	Load 35 g	65	90	115
mmHg 70~79	6	2.17	2.26	2.48	3.00	9.97	24.24	35.38	50 .02
80~89	15	1.93	2.37	2.72	2.99	7.95	20.77	32.81	58.57
90~99	12	1.61	1.92	2.31	2.61	4.45	13.98	27.83	42.60
100~109	4	1.88	2.13	2.23	2.50	5.18	13.80	30.80	43.83
110~	5	1.70	1.98	2.25	3.02	4.04	11.92	23.78	39.88

Table. 4. Relation between Mean Blood Pressure and Ocular Ballistic Movement

Mean Blood Pressure	Total Cases	Ocular Ballistic Displacement μ				Ocular Ballistic Energy ×16⁻⁶Erg			
		Load 35 g	65	90	115	Load 35 g	65	90	115
mmHg ~99	13	1.91	2.12	2.48	2.84	10.89	20.33	34.96	52.56
100~109	13	1.78	2.06	2.28	2.69	7.18	18.59	32.62	57.78
110~119	7	1.84	2.14	2.89	3.06	4.13	17.06	30.11	47.28
120~129	4	1.90	2.20	2.45	2.75	4.53	13.85	33.35	44.23
130~139	3	1.70	1.87	2.13	2.73	4.63	12.03	16.80	35.67
140~	2	1.60	2.00	2.00	2.45	4.15	12.90	30.30	47.95

Table. 5. Relation between Heart Stroke Volume and Ocular Ballistic Movement

Heart Stroke Volume	Total Cases	Ocular Ballistic Displacement μ				Ocular Ballistic Energy ×10⁻⁶Erg			
		Load 35 g	65	90	115	Load 35 g	65	90	115
c.c. ~40.0	7	1.54	1.61	1.93	2.44	3.91	8.80	17.81	30.84
40~50.0	18	1.78	2.16	2.69	2.81	4.60	14.90	32.67	57.74
50~60.0	6	2.21	2.55	2.77	3.20	13.00	27.85	40.67	67.97
60~70.0	6	2.00	2.25	2.50	3.30	9.55	26.34	37.33	59.48
70~	2	2.15	2.25	2.70	2.65	8.55	22.35	42.30	50.00

Relation of the mean blood pressure to displacement and energy. Mean blood pressure is generally regarded to reflect sharply the condition of the arterial wall. Mean blood pressure is represented by the following formula, diastolic blood pressure+ 1/3 pulse pressure, results is shown in Table 4. The energy gradually decreases from 90 mmHg along with the increase in blood pressure. But the tendency is not so marked in this case (Fig.3).

Relation of the heart stroke volume to displacement and energy. To use the relation of blood flow and energy we have computed heart stroke volume by Sodeman's formula. The displacement and energy curve shows a peak at the point corresponding to a 50-60 heart stroke volume.

From the curve it would be considered, that as in the control group, the heart stroke volume, displacement and energy show parallel correlation within a certain range (Table 5).

Summary

The ocular ballistic movement and its kinetic energy stand in parallel correlation with the amount of loading applied to the corneal limb. Here we must consider the

property of ocular rigidity and retrobulbar tissue as a medium for the transmission of retrobulbar pulsation. The loading at the corneal limb naturally increases ocular rigidity, which naturally influences transmission of retrobulbar pulsation. As a next report (cases with retrobulbar disorders), change in the pulse-transmissibility of retrobulbar tissue has a large effect on ocular displacement and energy. Its influence is far larger, than ocular rigidity itself. When heavy loading is applied to the eye ball, the record shows large dispersion. It may be due to the emotional reaction of the patient, or irregularity of loading, and others.

So from the data of 4 step loading, the heaviest loading was omitted, and only the data which shows linear change was adapted. Fig. 3 illustrates the results. From these results it may be considered, that the changes of ocular ballistic movement by step up loading is not so clearly related to the blood pressure, it is rather affected by the property of the blood vessel itself, and is strongly affected by the blood flow.

Consideration

On the previous report and in this report the author has investigated the reactivity of the ocular antero-posterior movement under a certain amount of loading. This problem will be discussed from the view of circulatory physiology, because no information has been obtained sofar.

As stated above this ocular reaction is produced chiefly by retrobulbar arteries, especially the ophthalmic artery; studying the phenomenon from twe view points, namely the condition of blood which fills the blood vessel (blood pressure) and the condition of the blood-vessel (arterial wall itself), the author has succeeded to make it clear, that ocular ballistic movement may be closely related to the stage of arteriosclerosis and blood pressure. In the first place the author discussed the problem of the arterial wall. In this report the author has determined the change of a blood vessel by the observation of retinal arteries because the object group does not include patients with local circulatory disorders. In regard to the classification of hypertension the author has adapted both to Keith-Wagener's and Scheie's classification systems, because each of them has its own benefit. In Keith-Wagener's classification it is possible to differentiate the malignancy or benignancy of the hypertension, but the grading of the sclerosis is rather neglected. On the other hand, Scheie's classification is more suitable to see the relation of the arterial wall, as is well proved in the result of our experiment.

In this series a group of hypertensive stage patients was deliberately chosen as experimental object. It is not rational to include them in the hypertensive group, because their blood pressure is very labile and the differ much from that of hypertensive group. They were treated as intermediate group between normal and hypertensive group. In this group, the actual data showed rather large dispersion and its average value is approximate to that of the normal control group. The most important

finding in this experiment is, that the displacement and energy of the ocular ballistic movements are remarkably affected by the loading and sclerosis of blood vessels. The author should like to consider the matter for a brief moment.

When blood flows into the artery the response of the sclerotic wall is less than that of a normal one. Lets think a little more about the strain of the arterial wall in the transverse section. Provided that the longitudinal strain of the wall is negligible, and its transverse displacement dr its kinetic energy will be represented by the following formula

$$\text{Energy} \quad E=\int\int p\cdot r\cdot d\cdot\theta\cdot 1\cdot dr$$

The above formula

$$E=\int\int p\cdot r\cdot d\cdot\theta\cdot 1\,\frac{dr}{2\pi\cdot r\cdot 1} \quad \cdots\cdots\cdots\cdots(1)$$

(Young's elasticity quotient $E=\frac{dp}{dv}$, $dp=E\cdot dv$, therefore

$dv=\frac{dp}{E}$, $dv=2\pi\cdot r\cdot 1\cdot dr$, so $dr=\frac{dv}{2\pi\cdot r\cdot 1}$

Formula (1) is

$$=\int_{p_1}^{p_2}\int_0^{2\pi} p\cdot r\cdot d\cdot\theta\cdot 1\;\frac{dp}{2\pi\cdot r\cdot 1\cdot E}$$

$$=\int_{p_1}^{p_2}\frac{P}{E}\cdot dp$$

$$=\cdot\,{}^1/_2\cdot 1/E\cdot[p_2^2-p_1^2]$$

p_2···systolic blood pressute

p_1···diastolic blood pressure

Therefore its kinetic energy is inversely proportionate to Young's elasticity quotient, and is proportionate to $p_2^2-p_1^2$. Should E increase as a consequense of arteriosclerosis, the kinetic energy in the transverse direction decreases, actually the raise of p compensates it. However, when arteriosclerosis aggravates and diastolic blood pressure should raise, p_2-p_1 lessens and affords only insufficient conpensation. Thus the forward thrust of the eye ball decreases. This experiment has proved it.

As recognized by many researchers, the heart stroke volume of hypertensive patients is not necessarily large. If the receiving part for blood volume becomes larger, $E=\frac{dp}{dv}$ lessens, and as a consequence the value of E becomes greater.

Conclusion

Since the first report the author has observed ocular ballistic movements of hypertensive patients in various stages and reached the following conclusion.

1. The response of hypertensive patients is smaller, than that of a normal one.
2. The response shows linear decrease along with the grade of Scheie's classification.
3. Raise of diastolic pressure is accompanied by a decrease of response.

4. Within a certain range, the heart stroke stands parallel to ocular ballistic movement.

From above results, it has been noticed, that ocular ballistic movement is strongly affected by arteriosclerosis.

II. INFLUENCE OF THE ORBITAL MEDIUM UPON THE OCULAR BALLISTIC MOVEMENT.

In the 1st and 2nd report, the author has detailed the response of the ocular ballistic movement to ocular pressure loading, viz. (1) the relation of cardiac output to the response of a normal person, (2) the relation of ocular ballistic movement to the property of intraorbital blood vessels, and (3) the relation of ocular ballistic movement to blood pressure, which is considered as a factor related to the tension of the blood vessel.

In this report the author has investigated the nature of the retrobulbar medium which plays an important factor in the transmission of ocular ballistic movement produced by orbital vessels. For this purpose, on patients with exophthalmos, due to various causes experiments were performed.

Method of Experiment

The apparatus and method of the experiments were the same as in the 1st and 2nd reports.

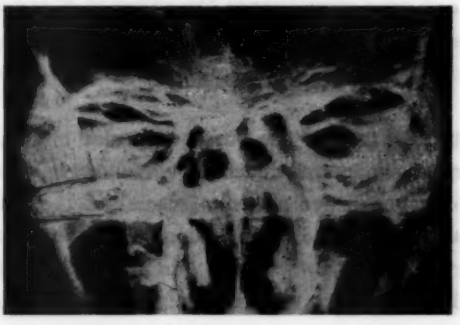
Experimental Materials

Five exophthalmos patients due to various causes were selected. They include one patient with orbital mucocele originated in the frontal sinus, one with maxillar cancer, one with pseud-orbital tumor, one with exophthalmos due to Grave's disease and one with experimental exophthalmos produced by retrobulbar injection of air.

Results

The details of each cases will be separately stated.

Fig. 1 Case (A). Mucocele of left frontal sinus.



Case A. Exophthalmos due to orbital mucocele.

Patient is 24 years old male, has noticed exophthalmos of the left eye 2 years ago, rightward movement of the left eye was restricted. Recently diplopia took place.

v.d,=1.5 (id.×+0.25 D) v.s.=0.4 (n.c.)

Degree of exophthalmos, right 16 mm, left 20 mm (by Hertel's exophthalmometer). Movement of the left eye was highly restricted. X-ray photograph is shown in Fig. 1. The examination of the fundus revealed a normal optic disc and slight degree of venous congestion. Before operation the ocular ballistogram of this patient disappeared by ocular pressure loading of middle degree.

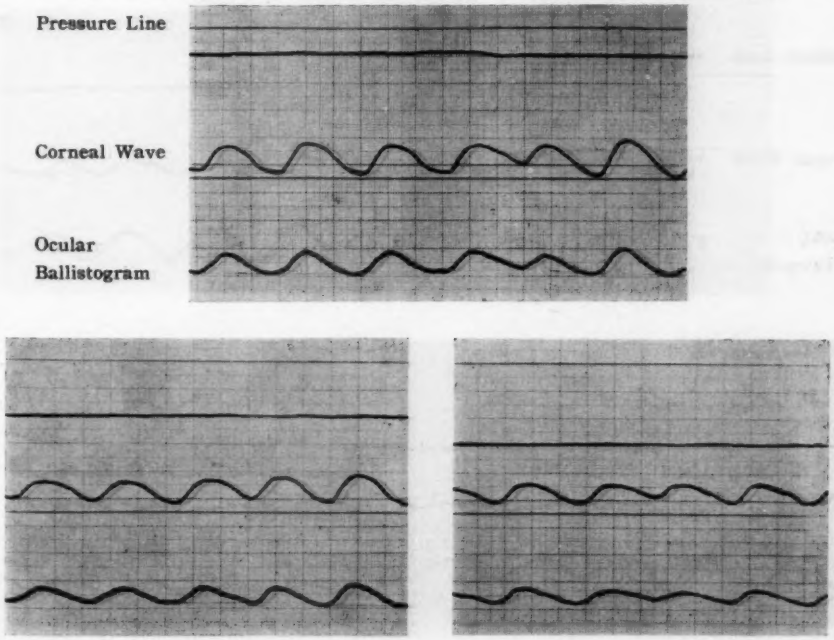
At the time of operation, it was found, that the mucocele in the left frontal sinus had grown up pretty large, and the sorrounding osseous wall was thinned out by its pressure.

7 days after the operation, the left exophthalmos decreased from 20 mm to 16 mm, and ocular movement improved. The ocular ballistic movement has recorded to the middle degree of loading and with light loading it shows almost the normal average (Fig. 2). Blood pressure 110-78.

Case B. Exophthalmos due to cancer of the maxillar sinus.

55 years old female, about one month ago the patient had noticed right exophthalmos and diplopia.

Fig. 2 Case (A). Mucocele of left frontal sinus. After 1 week of operation.



	Ocular Displacment μ			Ocular Ballistic Energy $\times 10^{-6}$ Erg		
	Load 35 g	65	90	Load 35 g	65	90
Before Operation	0.32	—	—	0.21	—	—
7 days after operation	1.67	1.57	1.04	6.0	11.31	7.93

Intraorbital pressure rises only in the right side, degree of exophthalmos, right 19 mm, left 14 mm (by Hertel's exophthalmometer).

Fig. 3 Case (B). Cancer of right maxillar sinus.

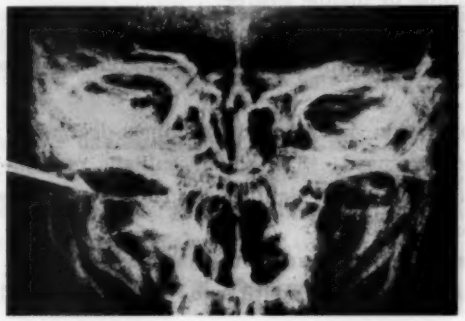
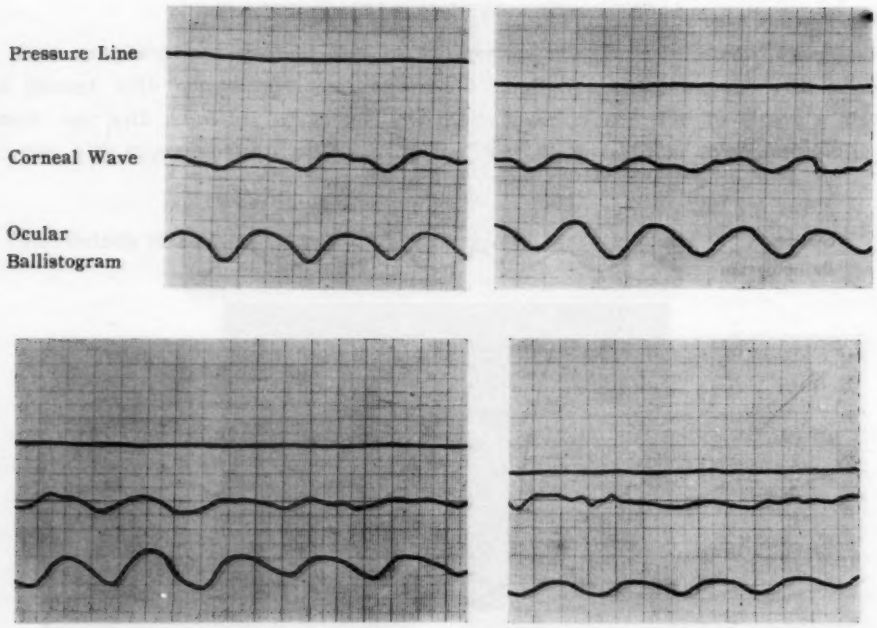


Fig. 4 Case (B). Cancer of right maxillar sinus.



v.d.=1.2 (n.c.) v.s.=1.2. (n.c.)

X-ray examination is shown in Fig. 3. The right eye was protruded antero-superiorly. The ocular movement was restricted. Diplopia was present. The blood pressure was 198-118. Retinal arteries were highly sclerotic.

The ocular ballistogram of this patient was low on the sound side. On the ill side displacement and energy were rather high when loading was light, it decreased rapidly as the weight of loading increased (Fig. 4).

	Ocular Displacement μ				Ocular Ballistic Energy×10⁻⁶Erg			
	Load 35 g	65	90	115	Load 35 g	65	90	115
Right	1.27	1.98	2.3	1.3	2.14	9.83	18.43	13.33
Left	2.25	2.25	1.76	0.93	7.39	13.73	10.8	5.82

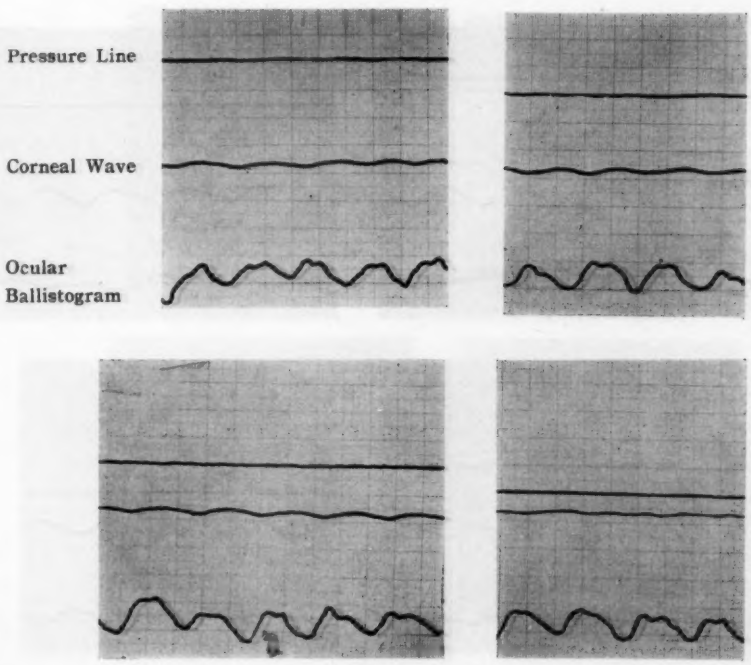
Case C. Exophthalmos due to Grave's disease.

26 years old female. The patient has noticed exophthalmos of both sides, and palpitation 8 months ago, and was diagnosed as Grave's disease by an internist.

v.d.=1.0 (1.2×+0.37 D) v.s.=1.0 (1.2×+0.37 D)

Exophthalmos: both sides 19 mm (by Hertel's exophthalmometer). Fundus: normal.

Fig. 5 Case (C). Grave's disease.



Ocular ballistogram revealed, that displacement and energy were extraordinarily large and increased remarkably by loading. Respiratory fluctuation was so large, that it was necessary to adapt the mean value of the respiratory and expiratory phase (Fig. 5). Blood pressure 116-62.

Ocular Displacement μ				Ocular Ballistic Energy ×10⁻⁴Erg			
Load 35 g	65	90	115	Load 35 g	65	90	115
19.2	24.4	26.0	29.0	1.14	4.0	5.27	7.74

Case D. Exophthalmos due to pseudo-orbital tumor.

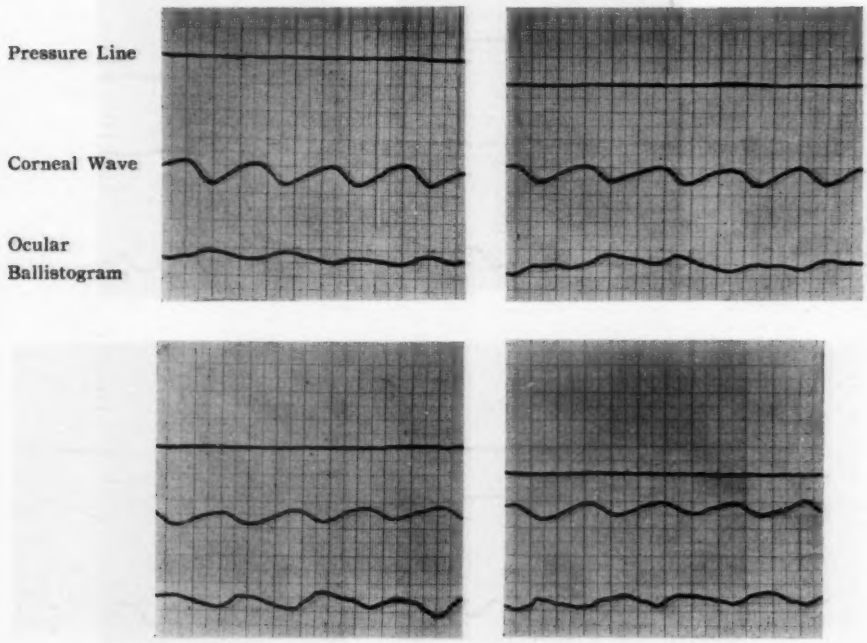
57 years old female. She underwent exstirpation of a right orbital tumor about 4 years ago. Exact callse of the disease was unknown. Visited the clinic for slight exophthalmos and ophthalmic pain.

v.d.=0.1 (0.7×−2.5 D) v.s.=0.5 (0.9×−1.75 D)

Relative centralscotoma in the right eye was noticed. Exophthalmos: right 16 mm, left 14 mm. A shadow was discovered in the right orbita by X-ray examination. Fig. 6 illustrates her ocular ballistogram. Increase of displacement and energy was noticed obviously in the right eye, compared with that of the left eye.

After administration of iodine for 2 months, the exophthalmos improved and the

Fig. 6 Case (D). Right pseudo-orbitaltumor.



ophthalmic pain disappeared. Blood pressure 164-98.

	Ocular Displacement μ				Ocular Ballistic Energy ×10⁻⁴Erg			
	Load 35 g	65	90	115	Load 35 g	65	90	115
Right	1.89	2.29	2.39	2.79	8.81	24.04	36.45	62.25
Left	1.65	1.96	1.91	2.43	6.72	15.47	20.2	48.31

Case E. Exophthalmos due to retrobulbar injection of air.
20 years old female.

Fig. 7 Case (E). After retrobulbar injection of air.

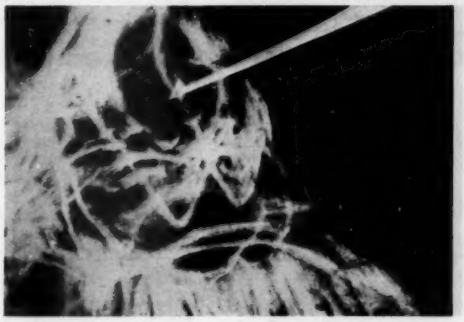


Fig. 8 Case (E). Before retrobulbar injection of air.

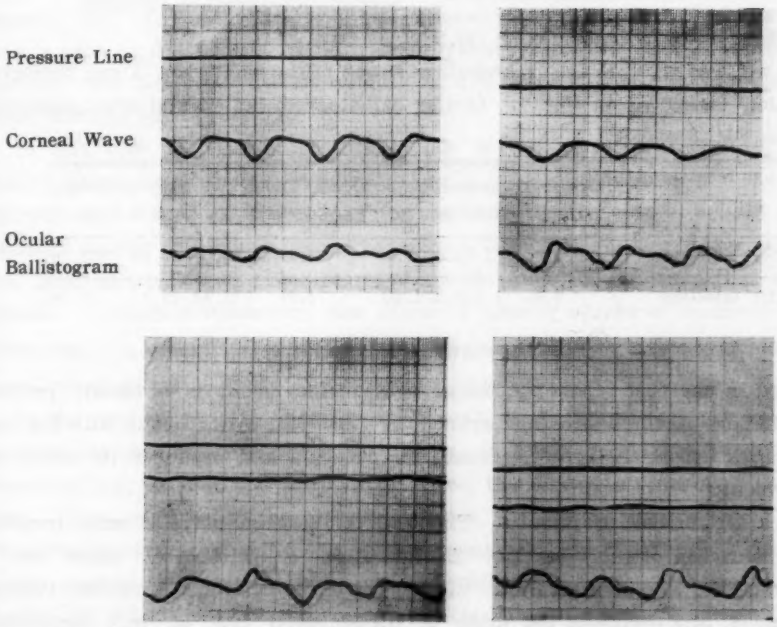
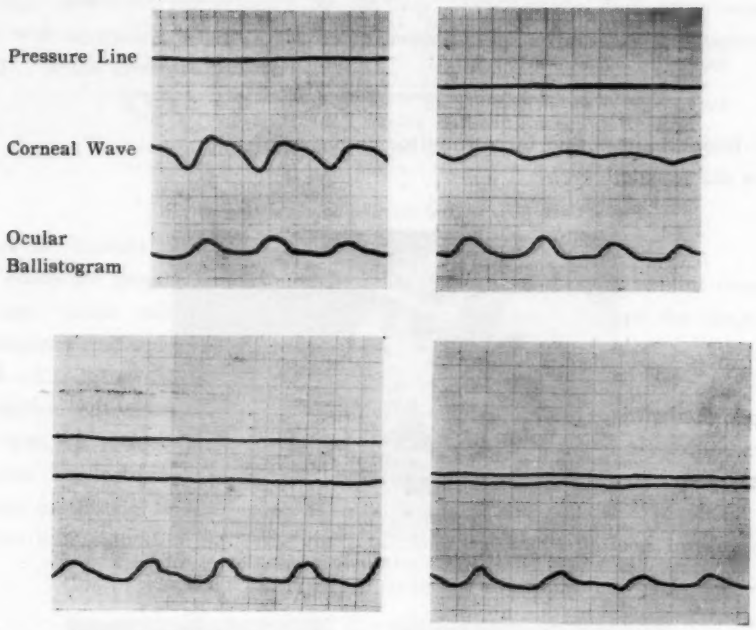


Fig. 9 Case (E). After retrobulbar injection of air.



v.d.=1.2 (id.×+0.25 D) v.s.=1.2 (id.×+0.25 D)

5 cc. of air was injected into retrobulbar tissue of the right eye. X-ray photograph after injection is shown in Fig. 7. Ocular ballistogram before and after injection is illustrated in Fig. 8 and 9.

	Ocular Displacement μ				Ocular Ballistic Energy $\times 10^{-6}$Erg			
	Load 35 g	65	90	115	Load 35 g	65	90	115
Before injection	2.97	3.5	3.68	4.2	9.68	24.60	54.45	112.76
After injection	1.05	1.14	1.32	1.26	4.99	12.42	19.6	19.34

Summary and Considerations

As stated in our first report the ocular ballistic movement is obviously produced by the pulsation of the ophthalmic artery, A vibration, synchronous with the heart stroke propagates from the artery, reaches the eye ball, and produces its antero-posterior movement.

This is a sort of ballistic motion. The kind of medium plays a very important role for transmission of the vibration. In this case the retrobulbar tissue and the eye ball serves the role of a medium. Living tissue, especially, retrobulbar tissue, is not homogenous, and therefore the mode of transmission may be very complicated.

The property of this medium as a whole, is regarded to show no great difference among normal individuals. The result of deformity of the eye ball due to pressuring of an intraorbital medium supports our opinion.

Therefore the property of the medium concerning ocular ballistic movement might be treated as developement of neoplasm or similar changes. By the theory of vibration this motion is represented by the vibration formula $X=ae^{-\lambda t}\sin(\omega t+\varphi)$, at the source of vibration in the antero-posterior axial plain. This formula offers a problem. λ is a quantity, which is closely concerned with the resistance against the vibration object.

Differentiating above formula $\frac{d^2x}{dt^2}+2\lambda\frac{dx}{dt}+(\lambda^2+\omega^2)x=0$ providing $c/m=2\lambda$, $k/m=\lambda^2+\omega^2$, $m\frac{d^2x}{dt^2}=-kx-c\frac{dx}{dt}$. $(-c\frac{dx}{dt})$ is resistance, which is in proportion to velocity. The main component of the plain, which meets this resistance is a problem, when viewed from the opposite direction.

From this formula it will be said, that the motive resistance of the medium (viz. volume elasticy, elastic resistance etc.) has a strong influence on the transmission of vibration.

The result of this experiment should be viewed on the basis of these ideas.

In the first place, on Grave's disease, many questions still exist as to the origin of the exophthalmos in this disease. According to Uemura's result of orbital tonometry, the eye ball gradually shows concavity as the pressure increases, no gap or jump is seen in this course. Therefore provided, that resistance is uniform, and its elasticity rate large, the transmissibility in this concern must be regarded to be high. These findings have proved it.

In this case the blood pressure is out of the question, therefore, taking into account Uemura's findings, the extraordinarily large displacement and energy is chiefly regarded to be due to the property of the medium. In the case of orbital pseudotumor a similar change to Grave's disease is seen. This time another factor, viz. hypertension, must be considered. The result of the ill side is larger, than that in a normal persons. Uemura's tonometry has shown a greater degree of concavity as result of pressuring. In other words, it means larger change of $\frac{\Delta p}{\Delta v}$, namely elasticity rate. It is well thinkable from the above mentioned theoretical formula.

In the next place lets ponder over the mucocele, cancer ond air injection cases, in which the mediums are not homogenous. It these substances, namely mucocele, cancer and air, exist in the retrobulbar space, the vibration reflects or passes through them. Thus, contrary to the proceeding cases, various results are seen, in one case the vibration strengthened, in the other it weakened and in some was fully absorbed. In the case of air injection, however, a specific wave is recorded due to the property of air. Air which has a large compressibility when compressed, shows the nature of fluid, and then as the pressure rises, takes the nature of a rigid body. Consequently

its initial motion is slow, accelerates rapidly and then quickly returns to its original state when the pressure is removed.

Shortly summed up, providing, that the source of antero-posterior movement, namely the artery be uniform, the medium plays a very important role, and by examining the antero-posterior movement of the eye ball, it will be possible to detect the change of the intraorbital medium—its homogenity, localization change, and rigidity etc.

Conclusion

Ocular ballistic movement produced by the pulsation of intraorbital blood vessels was investigated from the view of the kind of medium which contributed to transmit the pulsation. For the purpose to elucidate the various causes exophthalmos (orbital mucocele, cancer of the maxillar sinus, pseudo-orbitaltumor, Grave's disease and experimental retrobulbar injection of air), the experiments were performed. The apparatus and method of the experiments were the same as in the 1st and 2nd reports.

In regard to ocular ballistic movement, displacement and energy;

1. Grave's disease and pseudo-orbitaltumor had the maximum value aud showed a very high value, compared with the average of normal cases.
2. Cancer and mucocele took the 2nd place.
3. The case of retrobulbar injection of air had the least value. The value became low, compared with normal value which was measured prior to the injection.

Above results were compared with those of literatures, which were obtained by orbitonometry. Consequently, it is surely proved that the nature of retrobulbar medium plays an important factor on the transmission of ocular ballistic movement. Therefore, it is noticed, that ocular ballistography gives a clue to decide the diagnosis of intraorbital diseases.

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Correction

In the article "Experimental studies on the action potential of the iris" by Dr. M. Konno in the July~September issue, 1960, two figures (A and B) of Fig. 5 were accidentally inverted, and the next sentence "resistance placed in the primary circuit 16 Ω" was left out following the term "15 Ω" in the explanation of the same figures (page 198).

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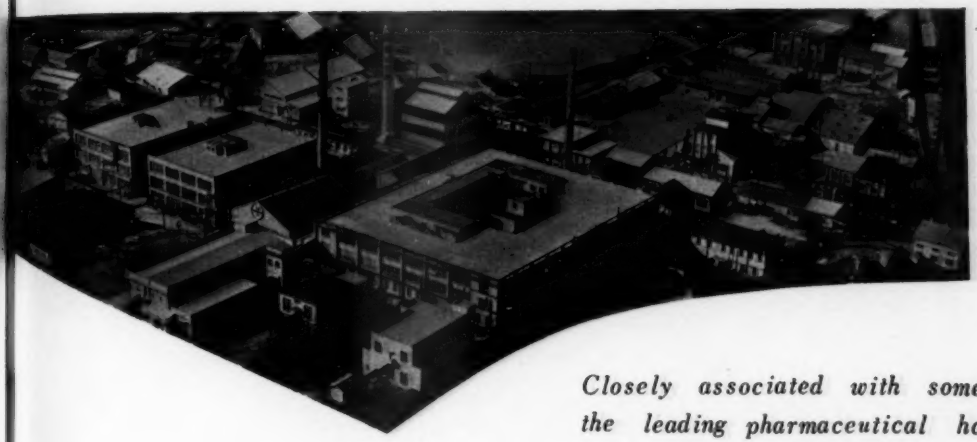

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